



UNIVERSITY OF
BIRMINGHAM

A CRITICAL EVALUATION OF THE UTILITY OF SUBCRITICAL
WATER TO SUPPORT THE PRODUCTION OF BIODIESEL AND
RENEWABLE DIESEL FROM THE LIPID FRACTION OF
ACTIVATED SLUDGE

BY

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ABSTRACT

This work investigates the use of subcritical water to support the production of biodiesel and renewable diesel from activated sludge derived lipids. The activated sludge slurry was collected from two sewage works in the United Kingdom at different seasons. The biomass content of the slurry was obtained by filtration and dried to a constant weight. The results showed that total biomass content in the activated sludge slurry was dependent on its source and seasons collected. The lipid yields obtained using organic solvents were 2.8 - 7.4 wt. %. The highest yield of FAMEs from acidic methanolysis of lipid extract was 13.9 (wt. %) or 0.4 (wt/wt. %, on dry activated sludge basis), with palmitic acid as the predominant fatty acid. The low yield of the lipid extract increased under subcritical water treatment and optimisation using Design of Experiment (DoE) to 41.0 (wt/wt) %. The lipid extract was further investigated for potential use as a feedstock for renewable diesel production, by catalytic hydrothermal decarboxylation. The maximum yields of the primary hydrocarbon products pentadecane and heptadecane from fatty acids: palmitic, oleic and stearic acids were 23.2% and 15.2%, respectively, obtained after 1 h hydrothermal decarboxylation in the presence of 5% Pt/C.

DEDICATION

This work is dedicated to the:

Almighty God

And

Nnama's family, especially my parents and Mr. Emeka Nnama for their unconditional love

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NOMENCLATURE

BOD	Biochemical oxygen demand
COD	Chemical oxygen demand
TOD	Total oxygen demand
TOC	Total organic carbon
RNA	Ribonucleic acid
DNA	Deoxyribonucleic acid
NPDES	National Pollutant Discharge Elimination System
PAH	Polycyclic aromatic hydrocarbons or polyhydroxyalkanoates
AD	Anaerobic digestion
EU	European union
PE	Phosphatidylethanolamine
PC	L – α - phosphatidylcholine
PS	1,2-diacyl-Sn-glycero-3-phospho-L-serine
PI	PI = L – α – phosphatidylinositol
MAG	Monoacylglycerol
TAG	Triacylglycerol
DAG	Diacylglycerol
FFA	Free fatty acid

HMA	Hexane/methanol/acetone
THF	Tetrahydrofuran
SCF	Supercritical fluid
SFE	Supercritical fluid extraction
SC-CO ₂	Supercritical CO ₂
PLFAs	Phospholipid fatty acids
TLC	Thin layer chromatography
HPTLC	High performance thin layer chromatography
GC	Gas chromatography
GHG	Greenhouse gases
FAMEs	Fatty acid methyl esters
SCW-SCM	Subcritical water – subcritical methanol
DoE	Design of Experiment
CCD	Central composite design
FCCCD	Face centered central composite design
RSM	Response surface methodology
ANOVA	Analysis of variance
OFAT/OVAT	One-factor-at-a-time/one-variable-at-a-time
STW	Sewage treatment works
TSS	Total suspended solid

TDS	Total dissolved solid
TS	Total solid
DS	Dissolved solid
BOC	British Oxygen Company
FID	Flame ionization detector

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2. World Academy of Science, Engineering and Technology (WASET): Certificate of Presentation, for oral and technical presentation, recognition and appreciation of research contributions to ICBB 2015:17th International Conference on Biofuels and Bioenergy, Paris, France
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THESIS LAYOUT

This thesis is presented in six chapters. The introduction and literature review are contained in Chapter 1. Here, a brief background of the project was given, followed by a presentation on wastewater, sludge, lipids, biofuels and Design of Experiment, as obtained from the literature, and lastly, the objectives of the project. Chapter 2, presents the materials and methods, starting with a brief introduction, followed by protocols on the characterization of activated sludge, lipid extraction, fatty acid methyl esters (FAMES) production, palmitic acid and vegetable oil recovery from water, subcritical water mediated lipid extraction from activated sludge, through design of experiment for the subcritical water mediated lipid extraction from activated sludge using face centred central composite design (FCCCD), production of renewable diesel and analytical methods were covered. Chapter 3 investigates the potential use of activated sludge as a feedstock for biodiesel, renewable diesel and oleochemicals production. The activated sludge biomass was characterised and lipid contents determined, and was further analysed. Chapter 4 assesses the utility of subcritical water to enhance the lipid content obtained from Chapter 3. It discusses also the optimisation of the lipid yield and solubilisation of the activated sludge biomass. Chapter 5 presents the potential use of the lipid extract from activated sludge for renewable diesel production through catalytic hydrothermal decarboxylation. Here, the effects of operating parameters: temperature and residence time on the performance of the reaction were investigated. Chapter 6, illustrates the conclusions and recommendations, wherein, inferences were made on the results presented in the work and possible works to be carried out in the future are shown.

1 CHAPTER 1 INTRODUCTION AND LITERATURE REVIEW

1.1 THESIS INTRODUCTION

Currently, about 80% of the world's energy requirement is produced from fossil fuels, of which 58% is consumed by the transport sector (Nigam and Singh, 2011). Consumption of fossil fuels results in the emission of greenhouse gases (GHG) which are thought to be driving climate change (Gullison et al., 2007). There has been a universal outcry arising from the imminent dangers of climate change and the need to reduce global warming. In response to both political and societal awareness of the potential impact of climate change, the Kyoto Protocol in 1997 and Paris Agreement (COP21) 2015 have attempted to develop roadmaps to forestall adverse effects by reducing the global warming to a temperature less than 2°C (EC, 2016, UNFCCC, 2008). It is widely understood that the development and adoption of renewable, sustainable alternative sources of energy can assist the mitigation of climate change. Biofuels either as liquid, gaseous or solid are seen as environmental-friendly energy sources (Kuye and Edeh, 2013). Over the last decade, there has been a rapid increase in biofuel production and currently accounts for 1.5% of the global transport fuels. (Eisentraut, 2010).

Biofuels such as biodiesel and renewable diesel are currently being investigated as substitute for the conventional diesel fuels (Kalnes et al 2009). Both biodiesel and renewable diesel are produced from fatty acids through thermochemical processes. The cost of feedstock accounts for 70 – 85% of the total biodiesel production cost (Hass and Foglia, 2005, Kargbo, 2010). thus there is a need for alternative source of feedstocks which have the potential to reduce the cost of production of biodiesel and renewable diesel. Lipids and fats sourced from waste and

residues have and continue to attract interest for example used frying oil, animal fats (Vyas et al., 2010). (Revellame et al., 2010). The following thesis is focused on exploring the potential of alternative sources of lipids derived from activated sludge (AS) which is a residue obtained from the biological section of wastewater treatment (Huynh et al., 2010). Activated sludge is principally made up of microbial flora, the growth of which has resulted in waste water purification, and therefore is a potential source of microbial lipids (Borchardt et al., 1977, Chipasa and Mdrzycka, 2008). Currently surplus activated sludge is mixed with sludge from primary and tertiary wastewater treatment sections and is called sewage sludge (Fytili and Zabaniotou, 2008, Manara and Zabaniotou, 2012, Metcalf and Eddy, 1991, Muhammad et al., 2002). The production of sludge is increasing globally due to the increasing quantity of wastewater generated and treated, resulting from rise in population, urbanization and industrialization (Yucheng and Arkur, 2012). Sludge production in Europe is estimated to be 90g per person per day, with a yearly production overage of 10 million tonnes (Davis, 1996). Therefore, with increased production of sludge there is a need to address disposal post waste water treatment. Currently there is considerable emphasis on the production of bioenergy from sludge, via gasification, combustion, and anaerobic digestion, the latter being able to accommodate wet biomass, with overall goal of supporting an energy neutral/closed loop waste treatment process (Fytili and Zabaniotou, 2008, Rulkens, 2008). However, gasification and incineration can result in toxic emission therefore the production of biodiesel and renewable diesel from lipids derived from sludge is seen as a complement to gaseous energy vectors or an alternative.

However, to obtain lipids/oil for the bioenergy production, it has to be extracted from the feedstocks. At commercial/industrial level, hexane is widely used to extract vegetable oil from oilseeds, but, it is toxic, and a volatile organic compound (VOC) which need to be managed to reduce environmental impact (Martinho et al., 2008, Temelli, 2009). At the laboratory scale,

lipids from biological materials are extracted using amongst other methods: Soxhlet, Folch, and Bligh and Dyer methods with organic solvents such as hexane, chloroform, ethyl ether, petroleum ether, toluene, methanol, ethanol and acetone (Boocock et al., 1992, Dufreche et al., 2007, Gungoren et al., 2007, Ju et al., 2000, Lili and Shunsheng, 2008, Pokoo-Aikins et al., 2010, Siddiquee and Rohani, 2011b).

In the light of the fact the use organic solvent often leads to potential health and environmental issues there is considerable interest in developing 'green' solvents which are deemed to environmentally benign and sustainable. Critical fluids such as carbon dioxide and water are widely recognised as green solvents and have and continue to be widely studied. For example supercritical carbon dioxide has been shown to be an efficient solvent for neutral lipid extraction and when modified with co-solvent such as ethanol can extract non-polar lipids (Dunford and Temelli, 1997, Sahena et al., 2009, Yamaguchi et al., 1986). As alternative to carbon dioxide subcritical water i.e. water maintained as liquid over the temperature range of 100 – 374 °C has been widely studied as solvent for the extraction of range compounds from biomass. Therefore comparatively, subcritical water is an attractive solvent for lipid extraction because it is nontoxic, non-flammable and inexpensive (Katritzky and Allin, 1996, Siskin and Katritzky, 2001) thus forms the focus of the research of this thesis.

In order to evaluate the utility of sub critical water as solvent for the extraction of microbial lipids from activated sludge and to support the subsequent transformation of the lipids to biodiesel and renewable diesel, there is need to optimise the process parameters. Adopting Design of Experiment (DOE), a statistical tool, multiple input factors to be manipulated determining their effect on a desired output (response). By manipulating multiple inputs at the same time, DOE can identify important interactions that may be missed when experimenting with one factor at a time. In addition, using DOE, significantly reduces the number of empirical experiments required to derive statistically relevant data to maximum lipid yield. Central

composite design (CCD) is commonly used to determine the interaction effects of factors, at more than two level designs, offers opportunity to explore the design region completely (Hibbert, 2012, Tixier et al., 2011). CCD includes rotatable, spherical, orthogonal quadratic and face centred designs. Face centred central composite design (FCCCD), has the advantages of allowing the use of less factors and it is less expensive (Anderson and Whitcomb, 2005, Ferreira et al., 2007) and hence was used in this project.

1.2 LITERATURE REVIEW

The following attempts to provide an overview of current wastewater management, the evolution of sludge types and current and potential routes of disposal, leading at the end to the section on the objectives of the thesis.

1.2.1 Wastewater

Municipal wastewater is the combination of liquid or water-carried wastes, originating from households (domestic residence), agriculture and commercial or industrial facilities, as a result of human activities, in addition to any ground water, surface water and storm water that may be present (United Nations, 2003, Perry and Green, 1999) (Figure 1-1). Municipal wastewater may be transported by trucks or through sewers to the wastewater treatment works. The flow and amount of wastewater handled by treatment works vary considerable according catchment area and based on these factors, no two communities can have exactly the same flow at the same time (Weiner and Mathew, 2003).

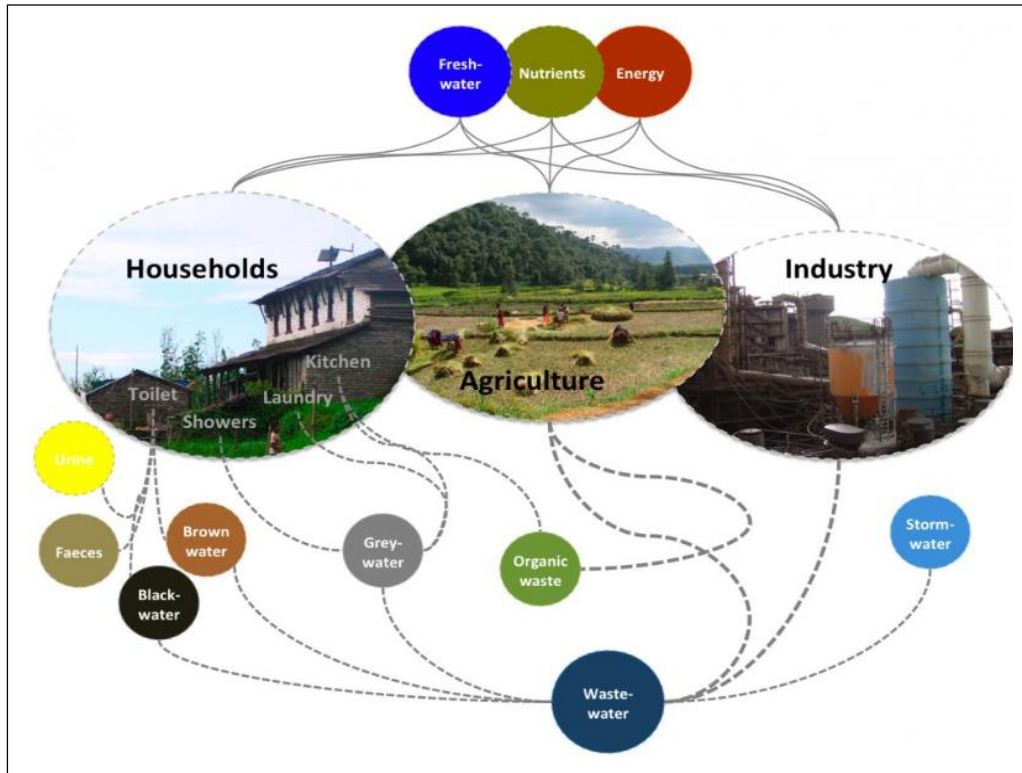


Figure 1-1: Sources of wastewater (Spuhler, 2010)

1.2.1.1 Types of wastewater

These are domestic (households), agriculture and industrial wastewaters (Figure 1-1). In this project, we have chosen to discuss domestic and industrial wastewater, since they are the most predominant source of wastewater to the treatment works where our samples were collected. Domestic wastewaters originate from homes, business premises and institutions and are referred to collectively as sewage (Templeton and Butler, 2011, Weiner and Matthew, 2003). The solid components may either be suspended or dissolved, the former can settle while the latter has to be converted to a settleable form, in order to enhance separation (Perry and Green, 1999). On the other hand, industrial wastewaters can be further classified as wastewater generated by iron and steel industries, mines and quarries, food, pharmaceuticals, chemicals and nuclear, thus the characteristics of industrial wastewater depends on its source. For instance, wastewater from food processing, dairy operations and meat packing would have a

high organic matter (high concentration of dissolved sugar, fat, protein and less amount of suspended matters) compared to those from plating industry, , hospital, etc. (Ragsdale and Associates, 2012).

1.2.1.2 Characteristics of wastewater

The quality of wastewater may be defined by its physical, chemical and biological characteristics (United Nations, 2003).

1. Physical characteristics

The physical characteristic of wastewater include colour, odour, temperature, turbidity, total solid content (floating and settleable matters, and colloidal particles in solution), oil and grease.

2. Chemical characteristics

The chemical properties of waste water are defined as biochemical oxygen demand (BOD), chemical oxygen demand (COD), total oxygen demand (TOD), total organic carbon (TOC), salinity, hardness, pH, acidity and alkalinity, as well as concentrations of ionized metals, such as iron and manganese and anionic entities such as chlorides, sulfates, sulfides, nitrates and phosphate.

3. Biological characteristics

The bacteriological parameters include the presence of coliforms, faecal coliforms, specific pathogens and viruses.

1.2.1.3 Treatment of wastewater

The objective of waste water treatment is to reduce the concentrations of particular pollutants in wastewater to a level, which when the effluent is discharged will not constitute any threats to health or environment (Weiner and Matthew, 2003). Therefore waste water treatment

reduces odour that usually emanates from untreated wastewater while also removing biodegradable organic matter, recalcitrant xenobiotics (example, pesticides, surfactants, phenols and priority pollutants such as benzene and chlorinated compounds), toxic metals (example cadmium, mercury and lead), suspended solid, nutrients (nitrogen and phosphorus), and microbial pathogens and parasites (Khopkar, 2004, Robert, 1999). Wastewater treatment ultimately generates clean water and organic matter that depending on its origin can be used as fertiliser on agricultural land, although there is increasing restriction on this route of disposal (Templeton and Butler, 2011, Vinay et al., 2011).

1.2.1.3.1 Wastewater treatment processes

Wastewater processing can be broken down into four stages, preliminary, primary, secondary and tertiary (advanced). The preliminary stage involves the removal of floating materials (such as rags, debris, etc.), large solids, abrasives grit, oil and grease. These materials are removed using physical unit operations such as screening, gritting, comminution and flotation (United Nations, 2003, Perry and Green, 1999, Weiner and Matthew, 2003).

The primary stage is designed to remove the suspended solids through the use sedimentation tanks or primary clarifier (Kent, 1992). and produces effluent with high energy molecules such as carbohydrate, protein and lipid which can be decomposed by microbial actions (Weiner and Matthew, 2003), see Figure 1-2.



Figure 1-2: a typical wastewater treatment process (1 = preliminary, 2 = primary, 3 = secondary and 4 tertiary wastewater treatment stages) (Media Age, Lentikats Biotechnology)

The third stage in wastewater treatment process is the secondary or biological stage (Figure 1-2) in which a microbial flora or activated sludge, removes soluble and colloidal organics, and suspended solids that might have escaped during the primary treatment (United Nations, 2003 Perry and Green, 1999, Kent, 1992). Activated sludge processes are preferred to trickling filter probably because, the microorganisms can be reused and its capacity to stimulate bacterial growth (Templeton and Butler, 2011). The activated sludge microorganisms include gram-negative bacterial (such as carbon and nitrogen oxidizers) and protozoa include flagellates and amoebas (United Nations, 2003). Bacteria are the most predominant microorganisms in the biological section of wastewater treatment plant, they are made up of between 70 and 85% water with solid matter consisting of protein (50%), cell wall (10-20%), ribonucleic acid (RNA) (10-20%), deoxyribonucleic acid (DNA) (3-4%) and lipid (10%) (Borchardt et al., 1977). Hence, surplus activated sludge is a potential source of lipids.

The fourth stage in wastewater treatment processes is the tertiary stage where nutrients such as nitrogen and phosphorus are removed (Dawei Han, 2012). The nitrogen can be eliminated by

nitrification or denitrification process and phosphorus is removed using biological or chemical processes (Han, 2012).

1.2.1.3.2 Sewage sludge

Sewage sludge is a residue of the primary, secondary and tertiary wastewater treatment processes (Section 1.2.3.1) and has high organic content (Fytili and Zabaniotou, 2008, Goyal et al., 2008, Manara and Zabaniotou, 2012, Metcalf and Eddy, 1991, Muhammad et al., 2002, Romdhana et al., 2009). The global annual production of sludge is increasing due to the demand for wastewater treatment, as result of rising population, urbanization and industrialization (Yucheng and Arkur, 2012). In the Europe, the increase in sludge production can also be attributed to the implementation of the Council Directive 91/271/EEC of 21st May 1991 on urban wastewater treatment (OJL, 1991). were in terms of per capital production it is estimated to be 90g per person per day, with an annual production exceeding 10 million tonnes (Davis, 1996). In China, 6.25 million tons (dry weight) was produced in 2013 and there was a total annual increase of 13% from 2007 to 2013 (Yang et al., 2015).

1.2.1.3.2.1 Sludge characterization

The characteristic of sludge is used to monitor the effectiveness of the wastewater treatment process and directing downstream sludge handling (Huynh et al., 2010). and is done using a wide range of laboratory methods. (Huynh et al., 2010, Spellman, 2009). Apart from identification of the predominant types of bacteria the analysis of the chemical constituents of the sludge such as nutrient is very important. (Table 1-1) as it will ensure compliance with the plant's National Pollutant Discharge Elimination System (NPDES) and will reveal sludge conditions and warns against impending process problems (Huynh et al., 2010). Moreover, the value of sludge as fertilizer is dependent on the availability of nitrogen, phosphorous, and

potassium, as well as trace elements (Weiner and Matthew, 2003). Finally, the chemical analysis enables the plant operator to determine the concentration of the heavy metals and other toxic substances which should be kept out of the food chain and general environment (Weiner and Matthew, 2003).

Table 1-1: typical chemical composition and properties of untreated/digested sludge (Metcalf and Eddy, 1991)

Parameter	Untreated primary		Digested primary		Activated range
	Range	Typical	Range	Typical	
Total dry solids (TS),%	2.0 - 8.0	5.0	6.0 -12.0	10.0	0.83 - 1.16
Volatile solids (% of TS)	60 - 80	65	30 -12.0	40	59 - 88
Ether soluble	6.0 - 30.0	-	5 - 20	18	-
Ether extract	7.0 - 35.0	-	-	-	5 - 12
Protein (% of TS)	20.0 - 30.0	25	15 - 20	18	32 - 41
Nitrogen (N, % of TS)	1.5 - 4.0	2.5	1.6 - 6.0	3.0	2.4 - 5.0
Phosphorous (P ₂ O ₅ , % of TS)	0.8 - 2.8	1.6	1.5 - 4.0	2.5	2.8 - 11.0
Potash (K ₂ O, % of TS)	0.0 -1.0	0.4	0.0 - 3.0	1.0	0.5 - 0.7
Cellulose (% of TS)	8.0 - 15.0	10.0	8.0 -15.0	10.0	-
Iron (not as sulfide)	2.0 - 4.0	2.5	3.0 - 8.0	4.0	-
Silica (SiO ₂ , % of TS)	15.0 - 20.0	-	10.0 - 20.0	-	-
Alkalinity (mg/l as CaCO ₃)	500 -1500	600	2500 - 3500		580 - 1100
pH	5.0-8.0	6.0	6.5 - 7.5	7.0	6.5 - 8.0

(-) not available

1.2.1.3.2.2 Types of sludge

Sludge is obtained from each stage of wastewater treatment processes, but, primary and secondary sludge are discussed as they contain high organic materials compared to those obtained from the preliminary and tertiary sections.

(1). Primary sludge

Primary sludge is made up of derived from the bottom of the clarifier in the primary stage of wastewater treatment processes (Kargbo, 2010). Basically, it is granular, grey material which contains organic and inorganic particulates (Robert, 1999, White and Plaskett, 1981).

(2). Secondary sludge or activated sludge

Secondary sludge or surplus activated sludge contains microorganisms, colloidal particles, organic polymers, mineral particles and cations, and is obtained from the biological wastewater treatment (Metcalf and Eddy, 2002). Typically, it is brown in colour, and flocculates (Templeton and Butler, 2011). The microflora is seen a potential source of lipids and has the potential as an alternative raw material for biodiesel production (Huynh et al., 2010). As mentioned earlier, activated sludge process is the most commonly used biotreatment processes for treating municipal and industrial wastewater (Mittal, 2011). It involves the aeration of the effluent from primary treated wastewater and acclimated microorganisms in the aeration tank for 6 – 8 h (Guyer, 2011), see Figure 1-2. The aerated mixed liquor flows under gravity or is pumped to the secondary clarifier where the flocculent activated sludge is separated from wastewater. The supernatant is pumped to the tertiary treatment section while a portion of the activated sludge is recycled back to the aeration tank and the surplus is supplied to the sludge handling section where it is mixed with the primary sludge for onward treatment and disposal.

1.2.1.3.2.3 Sludge treatment

Sludge from the primary and secondary clarifier's is usually subject to number of treatment steps i.e. dewatering, odour and pathogen reduction to minimise of disposal cost and ensure that the directives (e.g. Council Directive 91/271/EEC, Article 14 and the Council Directives 86/278/EEC) on disposal of sludge are adhered to, see Figure 1-3 (Templeton and Butler, 2011).

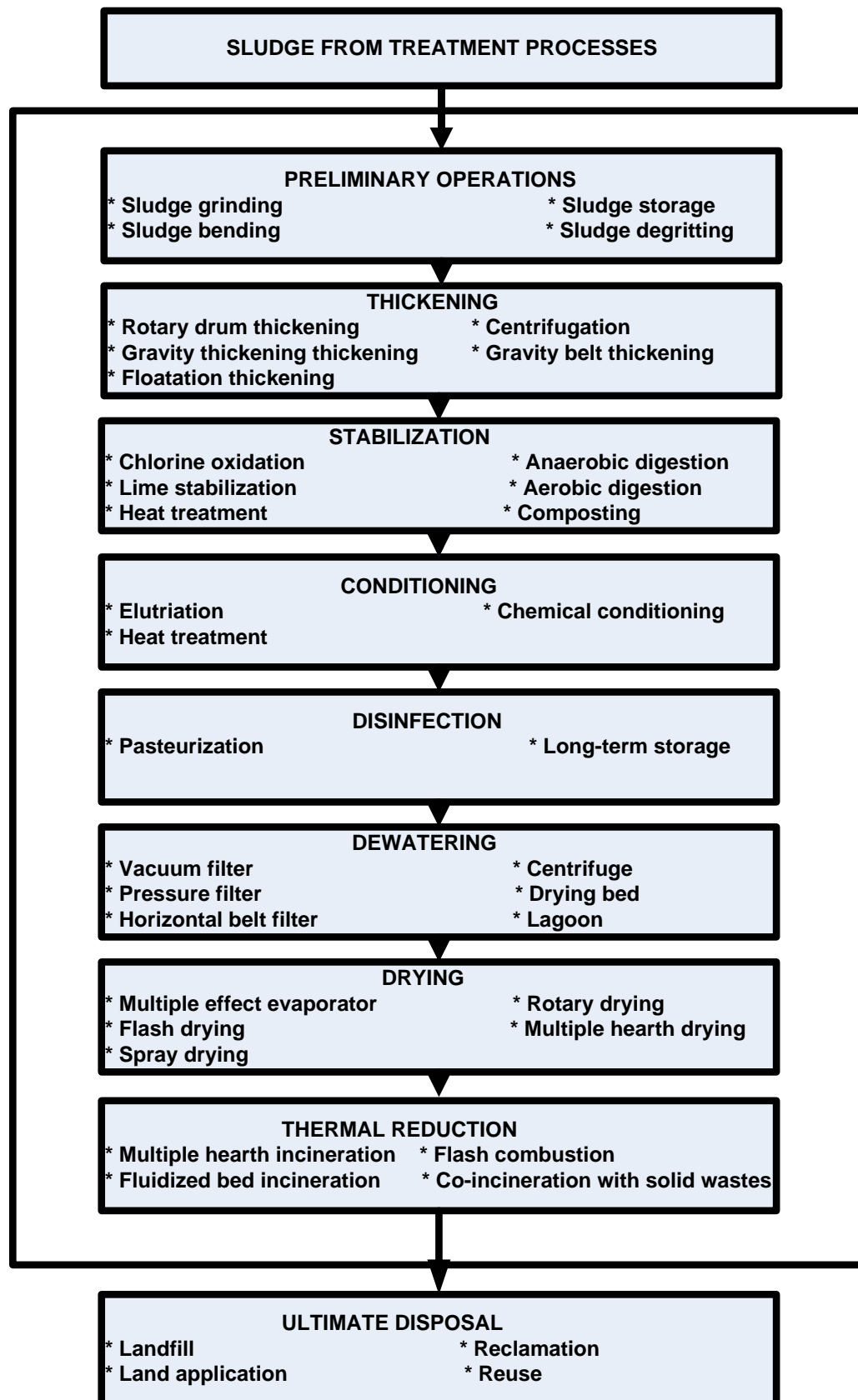


Figure 1-3: a generalised flow diagram for sludge treatment and disposal (United Nations, 2003)

1.2.1.3.2.4 Sludge disposal

Sludge for disposal is usually a combination of primary sludge and activated sludge which are thickened in order to reduce the cost of transportation (White and Plaskett, 1981). Sludge is usually disposed in the landfill, used as fertiliser on agricultural land reclamation or incinerated (Muhammad et al., 2002). These methods of sludge disposal subject to environmental restrictions. As a result of increased legislation and associated increased cost of disposal there is a growing interest in recovering value from the sludge via combustion, pyrolysis, and or anaerobic digestion, which leads to energy vectors which in turn can be used for electricity generation (Fytili and Zabaniotou, 2008, Rulkens, 2008). Brief descriptions are given in Sections 1.2.1.3.2.5 and 1.2.1.3.2.6

1.2.1.3.2.5 Routes to adding value to sewage sludge

(1). Pyrolysis of sewage sludge

Pyrolysis is a means of converting sewage sludge, once dried, into heat and bio oil (Inguanzo et al., 2002). It involves the thermal treatment of sludge under pressure at a temperature of 350 – 500°C in the absence of oxygen (Stolarek and Ledakowicz, 2001). The products of pyrolysis of sludge are gases (hydrogen, methane, carbon dioxide and carbon monoxide), liquid (tar and/or bio oil, acetic acid, acetone and methanol) and solids (char particle and some amount of inert substances) (Bien, 2007, Casanova et al., 1997, Fytili and Zabaniotou, 2008). The combustion of gaseous and liquid products derived from pyrolysis may lead to the emission of gases containing harmful substances such as polycyclic aromatic hydrocarbons (PAH) (Inguanzo et al., 2002, Zhang et al., 2013).

(2). Sludge gasification

Gasification is a thermochemical process which involves the conversion of dried sludge into ash and combustible gases (hydrogen and carbon monoxide) using oxygen ratio such as 0.12 to 0.27 at a temperature of 1000°C (Dogru et al., 2002, Fytili and Zabaniotou, 2008, Jaeger and Mayer, 2000, Werle, 2015). Like incineration, gasification can be used to reduce the volume of sludge generating an ash which can itself find application in brick manufacture for example although, differs in the emission of sulphur oxides, nitrogen oxides, heavy metals and the possibility of producing chlorinated dibenzodioxins and dibenzofurans (Marrero et al., 2004).

(3). Sludge combustion

Combustion/incineration is a chemical reaction between fuel (biomass) and oxygen to produce carbon dioxide and water with the emission of heat (Abdeshahian et al., 2010). Direct combustion has the advantages of producing lower sulphur emission (0.05-0.2 wt%) and better control of particulate formation in the sludge (Lee et al., 2007). The disadvantages of combustion include emission of pollutants such as heavy metals, ash, dioxins and furans which are dangerous to public health (Fytili and Zabaniotou, 2008, Werther and Ogada, 1999).

(4). Anaerobic digestion

Anaerobic digestion (AD) is a biological process where organic materials in the absence of oxygen is converted to biogas by the relevant microflora (Debruyne and Hilborn, 2007). The advantages of AD include the ability to process wet biomass, reduction in sludge volume and improved dewatering property of digested sludge (Şahinkaya and Sevimli, 2013). While its disadvantages include high capital and operational cost (Monnet, 2003).

(5). Hydrothermal gasification

Hydrothermal gasification involves the production of bio syngas (syngas – hydrogen and carbon monoxide; methane and carbon dioxide) from sludge in an aqueous medium at a temperature above 350°C and pressure of 20 MPa) and organic substances soluble in supercritical water (Basu, 2010, Boukis et al., 2004, Knezevic et al., 2009, Yokoyama, 2008). The advantages of hydrothermal gasification over a dry process, include high gas yield, low yield of tar/coke and it is more economical, as sludge with up to 80% (w/w) water content could be used (Kruse, 2009). The disadvantage is that it requires a high pressure vessel that can withstand high pressure and temperatures of 600°C or above (Kruse, 2008, 2009).

1.2.1.3.2.6 Comparison of the methods of creating useful products from sewage sludge

Some of the advantages and disadvantages of method of creating useful products from sewage sludge are presented in Table 1-2.

Table 1-2: advantages and disadvantages of methods of creating useful products from sewage sludge

Method	Advantages	Disadvantages
(1).Incineration	<ul style="list-style-type: none"> (i) high reduction of sludge volume by about 90% (ii) nearly complete elimination of the organic materials (iii) possible utilization for the ashes Obtained 	<ul style="list-style-type: none"> (i) incineration process can be energy deficient (ii) air pollution problems (NO_x and SO₂ emissions) (iii) dewatering/thickening of the sludge is required (iv) emission of chlorinated compounds (v) high cost due to the increasing demand on the flue gas cleaning
(2).Pyrolysis	<ul style="list-style-type: none"> (i) non-burning process (ii) production of a mixture of gaseous and liquid fuels and a solid inert residue (iii) conversion of all sludge biomass fraction into useful energy (iv) volume reduction by as much as 90 % and production of a sterile carbon char 	<ul style="list-style-type: none"> (i) dewatering/thickening of the sludge is required (ii) less technical maturity for its application to paper/pulp sludges
(3).Gasification	<ul style="list-style-type: none"> (i) high efficiency of energy recovery (ii) reduced environmental emissions (iii) ability to handle most inorganic compounds found in sludge (iv) production of an inert solid waste 	<ul style="list-style-type: none"> (i). dewatering and drying of sludge are needed
(4). Supercritical water oxidation	<ul style="list-style-type: none"> (i) easily controlled by operator (ii) reaction medium is water, so no dewatering/drying required (iii) high organic carbon destruction Efficiencies 	<ul style="list-style-type: none"> (i) corrosion and salt deposition in the equipment which accelerates the deterioration of the reactor
(5). Anaerobic digestion	<ul style="list-style-type: none"> (i) high energy recovery efficiency (ii) low operating temperature (iii) no dewatering/drying required 	<ul style="list-style-type: none"> (i) slow process, long residence times (ii) cannot accept shock loading and excessive foaming is often a problem

(Bermejo et al., 2006, Furness and Hoggett, 2000, Karayildirim et al., 2006, Kumar, 2000, Mahmood and Elliott, 2006, Monte et al., 2008)

1.2.1.3.2.7 Products extractable from sewage sludge

Some of the products which could be obtained from sewage sludge and their various applications are presented in Table 1-3.

Table 1-3: types of products recovered from sewage sludge and their uses (Kalogo and Monteith, 2008)

Type of product	Uses
Gases such as methane	electricity, heat and fuel
Oil, fat and grease	biodiesel and methane
Phosphorus and nitrogen	Fertilizers
Metals	Coagulants
Inorganic material	building material
Organic compounds	organic acid production

1.2.2 Lipids

There is no widely-accepted definition of lipids. However William Christie, a leading authority in lipid sciences, suggests that lipids are ‘fatty acids and their derivatives, and substances that are related to them functionally or biosynthetically’ (Christie, 2011b). Fatty acids are carboxylic acids with long aliphatic (hydrocarbon) chains (Christie, 2011a, Sahena et al., 2009) and are the prominent components of lipids and thus defined their physical, chemical and physiological properties (Ichihara and Fukubayashi, 2010). Fatty acids have an even number of carbon atoms in the range of C₈ to C₃₀, with C₁₆ and C₁₈ common in plants and animal tissues (Christie, 2011a). When there are only single bonds in between the carbon atoms, the fatty acid is said to be saturated while the presence of a double bond signifies that it is unsaturated and is

called polyunsaturated if there is more than one double bond see Table 1-4 (Christie, 2011a, Sahena et al., 2009).

Table 1-4: The common fatty acids of animal and plant origin (Christie, 2011a)

Systematic name	Trivial name	Shorthand	Chemical formula
Saturated fatty acids			
Ethanoic	Acetic	2:0	$\text{CH}_3\text{CO}_2\text{H}$
Butanoic	Butyric	4:0	$\text{CH}_3(\text{CH}_2)_2\text{CO}_2\text{H}$
Hexanoic	Caproic	6:0	$\text{CH}_3(\text{CH}_2)_4\text{COOH}$
Octanoic	Caprylic	8:0	$\text{CH}_3(\text{CH}_2)_6\text{COOH}$
Decanoic	Capric	10:0	$\text{CH}_3(\text{CH}_2)_8\text{COOH}$
Dodecanoic	Lauric	12:0	$\text{CH}_3(\text{CH}_2)_{10}\text{CO}_2\text{H}$
Tetradecanoic	Myristic	14:0	$\text{CH}_3(\text{CH}_2)_{12}\text{CO}_2\text{H}$
Hexadecanoic	Palmitic	16:0	$\text{CH}_3(\text{CH}_2)_{14}\text{CO}_2\text{H}$
Octadecanoic	Stearic	18:0	$\text{CH}_3(\text{CH}_2)_{16}\text{CO}_2\text{H}$
Eicosanoic	Arachidic	20:0	$\text{CH}_3(\text{CH}_2)_{18}\text{CO}_2\text{H}$
Docosanoic	Behenic	22:0	$\text{CH}_3(\text{CH}_2)_{20}\text{COOH}$
Monoenoic fatty acids			
<i>cis</i> -9-hexadecenoic	Palmitoleic	16:1(n-7)	$\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{CH}(\text{CH}_2)_7\text{CO}_2\text{H}$
<i>cis</i> -6-octadecenoic	Petroselinic	18:1(n-12)	$\text{CH}_3(\text{CH}_2)_{10}\text{CH}=\text{CH}(\text{CH}_2)_4\text{COOH}$
<i>cis</i> -9-octadecenoic	Oleic	18:1(n-9)	$\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{CO}_2\text{H}$
<i>cis</i> -11-octadecenoic	<i>cis</i> -vaccenic	18:1(n-7)	$\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{CH}(\text{CH}_2)_9\text{COOH}$
<i>cis</i> -13-docosenoic	Erucic	22:1(n-9)	$\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_{11}\text{COOH}$
<i>cis</i> -15-tetracosenoic	Nervonic	24:1(n-9)	$\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_{13}\text{COOH}$

Table 1-5: The common fatty acids of animal and plant origin (Christie, 2011a) (Contd.)

Polyunsaturated fatty acids*			
	Linoleic	18:2(n-6)	$\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7\text{CO}_2\text{H}$
6,9,12-octadecatrienoic	γ -linolenic	18:3(n-6)	$\text{C}_{18}\text{H}_{30}\text{O}_2$
9,12,15-octadecatrienoic	α -linolenic	18:3(n-3)	$\text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$
5,8,11,14-eicosatetraenoic	Arachidonic	20:4(n-6)	$\text{CH}_3(\text{CH}_2)_4(\text{CH}=\text{CHCH}_2)_4(\text{CH}_2)_2\text{CO}_2\text{H}$
5,8,11,14,17-eicosapentaenoic	EPA	20:5(n-3)	$\text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCOOH}$
4,7,10,13,16,19-docosahexaenoic	EPA	22:6(n-3)	$\text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCOOH}$

* all the double bonds are of cis configuration

1.2.2.1 Classification of lipids

Lipids may be classified as simple or complex lipids (Christie, 2011a). Simple lipids comprise two kinds of primary products per mole (example fatty acids, sterol and acylglycerols). While complex lipids comprise of three or more primary products (example glycerophospholipids and glycosphingolipids) (Christie, 2011a, Fahy et al., 2005). Lipids can also be classified as neutral and polar, but, this seems confusing as some lipids called polar might be more neutral and vice versa (Christie, 2011a). For instance, phosphatidylethanolamine (PE) is classified as a polar lipid whereas it is less polar than some neutral lipids while free fatty acids are usually referred to as neutral lipids whereas, they are more polar than most polar lipids (Xiao, 2010).

1.2.2.1.1 Simple lipids

Some of the simple lipids are presented below:

(i). **Triacylglycerols (triglycerides)**; are found in all fats and oils of animal and plant origin (Sahena et al., 2009) and are synthesized in nature by enzymes and consist of a glycerol moiety, with each hydroxyl group esterified to fatty acid, see Figure 1-4 (Christie, 2011a).

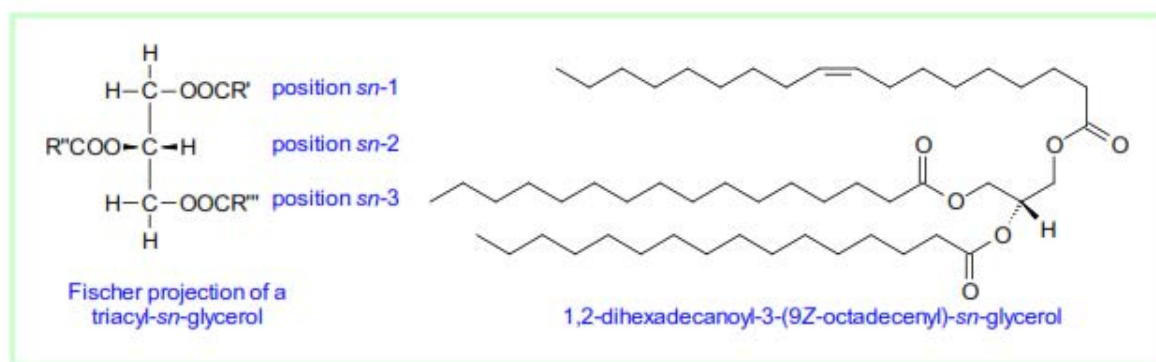


Figure 1-4: triacylglycerols (Christie, 2011a)

(ii). **Diacylglycerol (diglycerides) and monoacylglycerols (monoglycerides)**: Diacylglycerides (DAG) and monoglycerides (MAG) contain two and one mole of fatty acid per mole of glycerol, respectively (Christie, 2011a) and are found in trace quantity in fresh animal and plant tissues. Both DAG and MAG can be biosynthetic intermediates or derived from hydrolysis. For instance, the hydrolysis of phosphatidylinositol by phospholipase C produces 1, 2-diacyl-*sn*-glycerols and that of triacylglycerols results to 2-monoacyl-*sn*-glycerols which is used as surfactant, see Figure 1-5 (Christie, 2011a).



Figure 1-5: (a) Diacylglycerols and (b) Monoacylglycerols (Christie, 2011a)

(iii). **Waxes:** are simple lipids produced from the esterification of fatty acids to long-chain alcohols of similar chain-lengths (example wax ester) (Figure 1-6) found in animal, plant and microbial tissues (example bee honeycomb, carnauba, candelilla, sheep wool, ambergris and spermacetic of sperm whale (Christie, 2011a, Kolattukudy, 1976). For the microbial waxes, their composition depends on the types of nutrient, growth conditions and strains (Ishige et al., 2003). Waxes can be used for energy storage and for the production of cosmetics, lubricants, polishes, waterproof and surface coating (Huynh et al., 2011).

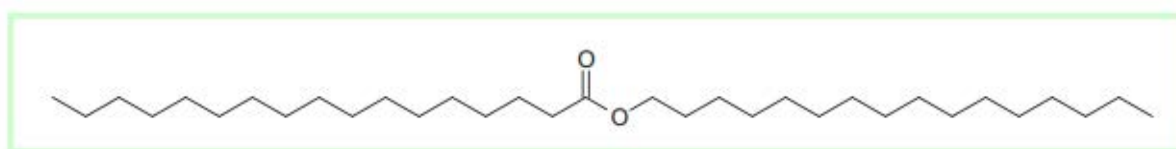


Figure 1-6: Waxes (Christie, 2011a)

(iv). **Free (unesterified) fatty acids:** these are fatty acids that are not attached to any molecules. They could function as source of energy and cellular messengers (Christie, 2011a).

(v). **Sterols and sterol esters:** are lipids which exist in both free and esterified states with cholesterol as the dominant steroids in animal tissues (Christie, 2011a).

1.2.2.1.2 Complex lipids

Complex lipids include glycerophospholipids (phospholipids) and glycolipids (which include glycoglycerolipids and glycosphingolipids) (Christie, 2011a). Phospholipids contain a polar

phosphorus moiety and a glycerol backbone while the glycolipids contain a polar carbohydrate moiety (Christie, 2011a).

(1). Glycerophospholipids (phospholipids)

Phospholipids are the major components of the cell membrane and include phosphatidic acid, lysophosphatidic acid, phosphatidylglycerol, cardiolipin, lysobisphosphatidic acid, phosphatidylcholine, lysophosphatidylcholine, phosphatidylethanolamine, N-Acylphosphatidylethanolamine, phosphatidylserine, phosphatidylinositol and phosphonolipid (Christie, 2011a).

1.2.2.2 Lipids available in activated sludge

The organic content of domestic wastewater contains about 60-70% of proteins and lipids (Raunkjaer et al., 1994). Lipids such as oils, greases, fats or long-chain fatty acids are important organic components of municipal and industrial wastewater (Becker et al., 1999, Henze, 1992, Quemeneur and Marty, 1994, Raunkjaer et al., 1994). These lipids, especially free fatty acid (FFA) are less responsive to biodegradation compared to other organic substrates such as sugars and amino acids. As a result of this, they tend to accumulate during biological treatment of wastewater (Chipasa and Mdrzycka, 2008, Novak and Kraus, 1973, Pei and Gaudy, 1971). The effect of which will be the direct adsorption onto the municipal sewage sludge (Kargbo, 2010). Thus, lipids in the activated sludge are attributed to the following, adsorption and accumulation of lipids during biological treatment of wastewater, cell wall component of bio-products from metabolites, the bio-transformation of a complex mix of organic matters in wastewater by microorganism communities and the phospholipids in the cell membranes of microorganisms (Chipasa and Mdrzycka, 2008, Quemeneur and Marty, 1994, Selmann and Holst, 2002, Siddiquee and Rohani, 2011b). The type of lipids that are mostly prevalent are the fatty acids, especially C₁₆ and C₁₈, which emanate commonly from vegetable oils or animal

fats from kitchen wastes and faeces (Quemeneur and Marty, 1994). Fatty acids also, result from the hydrolysis of lipids (glycerides) by extracellular enzymes (mainly lipase) of bacteria microorganism, this process also gives rise to free fatty acids (Dueholm et al., 2001, Hwu et al., 1998). Fatty acid constitute the bulk part, approximately 60% of the total lipids content of the sewage sludge (Higgins et al., 1982). However, according to Van Gerpen et al. (2004), after extraction and separation of the fatty acid from the bulk of the lipids, it is observed that its quantity is much greater than 1 wt% free fatty acid and as such, not fit for the production of biodiesel using alkali transesterification methods, due to possibility of soap formation (Kargbo, 2010). Thus, in order to utilize the fatty acid, a pretreatment is required to reduce the free fatty acid contents. Such pretreatments include enzymatic methods, acid catalysis, glycerolysis and acid catalysis succeeded by alkali catalysis (Van Gerpen et al., 2004). But acid catalysis has a potency of converting a high free fatty acid directly into methyl esters which can be processed to biodiesel (Van Gerpen et al., 2004).

1.2.2.3 Lipid/oil extraction

Lipids may be extracted using various methods such as Soxhlet, microwaves, accelerated solvent, and critical fluid extractions (Sahena et al., 2009). Solvent and critical fluid extractions are discussed.

1.2.2.3.1 Solvent extraction

1.2.2.3.1.1 Organic solvents used in lipids extraction

Organic solvents such as hexane, chloroform, ethyl ether, petroleum ether, toluene, methanol, ethanol and acetone have been used in extracting lipids from biological materials and can be combined together to enhance the efficiency of lipid extraction, for instance, chloroform-methanol, hexane-ethanol, dichloromethane-methanol (Boocock et al., 1992, Dufreche et al.,

2007, Gungoren et al., 2007, Ju et al., 2000, Lili and Shunsheng, 2008, Pokoo-Aikins et al., 2010, Siddiquee and Rohani, 2011, Zhu et al., 2012). The properties of solvents such as polarity, volatility, non-miscibility with water, boiling point, environmental factors, absence of toxic or reactive impurities, tendency to produce two phase system with water in order to remove the non-lipids substances, ability to extract a wide range of lipid classes, and cost have to be considered before they are selected for lipid extraction (Boocock et al., 1992, Hara and Radin, 1978). The polarities of solvents and lipids should correspond, this means that polar solvent should be used for polar lipid extraction and vice versa. For instance, methanol should be a good choice of solvent for polar lipids such as phospholipid and would not be effective in the extraction of a non-polar lipid, triglycerides or cholesterol esters, except if used with a co-solvent (Liu, 1994a). Safety and cost are also important parameters to be considered when making solvent selection. For instance, ethanol and methanol are very expensive but are the safest. Thus, in order to use them, a cost penalty of about 20% increase has to be incurred while the safety index will increase by 60%. Conversely, solvent such as toluene would have a high safety penalty at the expense of cost, as it is relatively cheap, but hazardous (Pokoo-Aikins et al., 2010).

1.2.2.3.1.2 Solvent extraction methods for laboratory purposes

These include Soxhlet, Folch, and Bligh and Dyer methods. Soxhlet is the most commonly used solvent extraction methods (Luque de Castro and Garcia-Ayuso, 1998). Its advantages include very easy to use, requires no filtration, inexpensive, greater potential of extracting more sample mass with respect to other methods and it encourages simultaneous and parallel extraction. While its drawbacks include long extraction time and demands large volume of solvents, health and environmental risks, potential of thermal degradation of sample and not easily automated due to solvent selectivity problems (Luque de Castro and Garcia-Ayuso,

1998, Naude et al., 1998). Factors that affect Soxhlet extraction include type and quantity of solvent, solvent-to-sample ratio (high), temperature, sample preparation, and extraction time (sufficient to ensure the dissolution of the sample). But, the most important of these factors is the type of solvent that is used for the extraction (Luthria, 2004). To use Soxhlet apparatus, solvent in the distillation flask is heated to the boiling point and the vapour passes through a tube to the reflux condenser where it is condensed. The condensed solvent vapour tickles down and fills the thimble-holder containing the sample. The soluble part of the sample gets dissolved in the condensed solvent vapour, as the liquid tickles down, until the liquid mark is reached. At this mark, the siphon aspirates the extracted analytes with the liquid content of the cellulose thimble into the distillation flask. The process continues automatically, until the stipulated extraction time is reached and assuming that a complete extraction is attained at this time (Luque de Castro and Garcia-Ayuso, 1998, Vogel, 1956).

Folch method (Folch et al., 1957) is another means of extracting lipids using organic solvents chloroform and methanol (2:1, v/v). It has been considered as a standard method for recovering and quantifying total lipids (Iverson et al., 2001). Similar to Folch method in solvent system and function, is the Bligh and Dyer method, although, the difference is that the latter uses less volume of organic solvents: 3 parts of chloroform/methanol (1/2, v/v) for 1 part of sample, compared to the latter and hence, more economical (Iverson et al., 2001).

1.2.2.3.1.3 Applications of solvent extraction

The uses of solvent extraction in vegetable oil and lipids production from oilseeds and sludge, respectively, are presented in this Section.

1.2.2.3.1.3.1 Industrial vegetable oil extraction

The extraction of lipids/oil from oilseeds (soybean, safflower, etc.) at an industrial level is performed using solvents, although, a percentage of oils for example olive oil is produced using a press (Han et al., 2009). Hexane is the solvent of choice for the extraction of vegetable oils at industrial scale as it is chemically stable, and supports efficient extraction. The vapour pressure of hexane enables distillation of the oils to ensure recovery of the solvent (Martinho et al., 2008, Pagliero et al., 2011). However, it is not possible to achieve 100% recovery of hexane, resulting in a very small percentage contamination of the product (Chan and Ismail, 2009, Han et al., 2009). Hexane is toxic, and thus, poses safety and environmental challenges leading to restrictions by government (Martinho et al., 2008, Temelli, 2009).

1.2.2.3.1.3.2 Lipid extraction from sludge (laboratory scale)

To date the extraction of lipids from water derived sludge remains at lab scale and there is continued research to determine the optimal method as many factor influence the overall efficacy for example type of sludge, solvent, extraction time, temperature, stirring rate, kind of microorganisms found in the sludge (Siddiquee and Rohani, 2011b). The following describes a number of studies designed to evaluate the impact of different organic solvents on the efficiency of lipid extraction from sewage sludge. Thus, Zhu et al. (2012) used the single solvents: bromo-propane, hexane, acetone, toluene, petroleum ether and mixed solvents: chloroform-methanol, and hexane-ethanol to determine the solvents, single or mixed which could give the highest yield of lipids from municipal sludge. They found that for the single solvents, bromo-propane gave the highest lipid yield, followed by ether, toluene, n-hexane and petroleum ether. While for mixed solvents, hexane-ethanol gave the highest lipid yield. Comparing the yields of both the single and mixed solvents, they concluded that mixed solvent produced the highest lipids extract. In terms of polar and non-polar solvents, they concluded

that the former, extracted more lipids because, the secondary sludge (microbial cells) contains more of the polar phospholipids than the neutral acylglycerols in the primary sludge.

Boocock et al. (1992) carried out two different extractions using chloroform and toluene and a boiling solvent extraction method, with solvent-to-feed ratio of 1 to 6. Using a warm 300 mL solvent and 50g of sewage sludge in a Soxhlet apparatus with about 11 min as the time taken to complete each siphoning cycle, they obtained a yield of 12 wt%. While using a boiling 600 mL chloroform or toluene and 100 g dried sludge they obtained 17 – 18 wt%. They concluded that chloroform and toluene are effective solvents for lipid extraction. Their results, also showed that approximately 65 wt% and 7 wt% of the extracts were free fatty acids and acylglycerols, respectively, constituting C₁₂ – C₁₈ with palmitic, stearic and oleic acids having a greater proportion.

Dufreche et al. (2007) used a 200 series accelerated solvent extraction system (Dionex, USA) to extract lipid from a dewatered activated sludge mixed with hydromatrix (water absorbent) using 60% hexane/20% methanol/20% acetone (HMA), pure methanol followed by pure hexane (MH), as the solvents. The conditions used were 10.3 MPa, 100°C, extraction time of 1h per each extraction and solvent-to-solids ratio of 40/1 (g/g). They obtained a maximum lipid yield of 27.43 ± 0.98 %, for three replicates, using the solvent mixture, HMA. Their results showed that sequential extraction using hexane, methanol, and acetone mix had a significant amount of unextracted materials after the first extraction and that the quantity of extractable oil decreased sharply with each subsequent extraction. They further showed that extraction with pure methanol gave 19.39% yield compared to 1.94% obtained with pure hexane, as polar lipids are extracted more easily using polar solvents with large values of δ_p (dipole moment contribution) and δ_h (hydrogen-bonding contribution) and hexane extracts nonpolar lipids with low values of δ_p and δ_h .

Furthermore, Gungoren et al. (2007) carried out Soxhlet extraction experiments for 8h on dried sludge to determine a suitable solvent for high recovery of extract. Their results showed that the extraction with tetrahydrofuran (THF) gave the highest yield of the extract of 30.6 wt% while the least was obtained using water, 7.5 wt%. They concluded that there was no appreciable difference in the yield of the lipids extracted with respect to the solvent-to-sludge ratio, 3, 1.5, and 1, investigated. Although, as a result of less volume of the solvent required when using a solvent-to-sludge ratio of 1, it is assumed as the optimum value.

Pokoo-Aikins et al. (2010) used four different solvents, toluene, hexane, ethanol and methanol to extract lipids through Soxhlet extraction and boiling extraction methods. For the Soxhlet extraction, they used 300 mL of the solvent and 50g of dried sewage sludge. While for the boiling extraction, they used 600 mL of the solvent and 100g of dried sewage sludge. Their results gave a maximum triglyceride yield of 3.4% in the entire processes. The yields of fatty acids were 24.8% and 24.9% using toluene and hexane, and 25.5% using either methanol or ethanol. They concluded that in view of economic considerations in terms of the order of preference that toluene > hexane > methanol > ethanol are used for lipid extraction from sewage sludge, but, for safety purposes ethanol > methanol > hexane > toluene are preferred.

Apart from the works presented above on lipid extraction from sludge, using organic solvents as carried out by different investigators, other studies are summarized in Table 1-6.

Table 1-6: Laboratory scale of lipid extraction from sludge

S/N	Extraction method	Lipids yield	Solvents	Feed	Extraction conditions	References
1.	Solvent extraction	<p>(i). <i>Using methanol</i></p> <p>(a). Primary sludge: 14.46 (wt/wt)%</p> <p>(b). Secondary sludge: 10.04 (wt/wt)%</p> <p>(ii). <i>Using hexane</i></p> <p>(a). Primary sludge: 11.16 (wt/wt)%</p> <p>(b). Secondary sludge: 3.04 (wt/wt)%</p>	Methanol and hexane	Primary and secondary sludge	Dried primary sludge = 5g; dried secondary sludge = 1g; solvent flash of 25 mL and 10 mL for lipid extractions from primary and secondary sludge, respectively.	(Siddiquee and Rohani, 2011)
2.	<p>(i). Solvent extraction without subcritical water pre-treatment</p> <p>(ii). Solvent extraction with subcritical water</p>	<p>(i). Neutral lipid: 2.10 (wt/wt)%</p> <p>(ii). Neutral lipid: 7.87 (wt/wt)%</p>	Sub-critical water and hexane	Activated sludge	<p>The sludge powder was mixed with diatomaceous earth (=95% SiO₂ basis – Sigma Aldrich). Moisture content of the sun-dried sludge and oven dried sludge were 9.22% and 2.60%, respectively.</p> <p>10g of sludge powder, 15 ml deionised water, reactor temperature and pressure were 175°C and 2 MPa, reaction time was 15 min</p>	(Huynh et al., 2010)
3.	<p>(i). Bligh & Dyer extraction (BDE)</p> <p>(ii). Accelerated solvent extraction (ASE)</p>	<p>(i). Partially dewatered sludge: 12.5 (wt/wt)%</p> <p>(ii). Freeze dried sludge: 12 (wt/wt)%</p> <p>12 (wt/wt)%</p>	<p>Methanol, chloroform and water</p> <p>n-hexane, methanol and acetone</p>	Activated sludge	<p>Concentrated sludge (8-16% weight solid), freeze-dried sludge, two times extraction, desolventization using Buchi R-205 rotary evaporator at 40°C and 300 mbar, and Turbo Vap LV using 45°C and 15 psi stream of N₂</p> <p>Freeze-dried sludge; weight of the sample = 4g; diatomaceous earth = 1g; volume of the extraction cell = 22ml; extraction temp. = 100°C; extraction pressure = 10.34 MPa; Solvent system = n-hexane/methanol/acetone = 60/20/20 (v/v/v); total extraction time = 1h; solvent flush = 75% of the extraction cell's volume</p>	(Revellame et al., 2012)
4.	Soxhlet extraction	<p>Primary sludge = 25.3 (wt/wt)%</p> <p>Blended sludge = 21.9% (wt/wt)%</p> <p>Stabilized sludge = 10.1 (wt/wt)%</p> <p>Secondary sludge = 9.1 (wt/wt)%</p>	Hexane	Primary sludge, blended sludge, stabilized sludge and secondary sludge	<p>Sludge pre-treatment; blended sludge was obtained by blending the primary and secondary sludge with ratio of 65:35; volume of hexane = 200 mL; 20g of sludge sample; 80 cycles of the extraction in approximately 5.5 h.</p>	(Olkiewicz et al., 2012)
5.	<p>(i). Solvent extraction (Bligh & Dyer method as modified by white et al. (1979))</p> <p>(ii). Supercritical CO₂ with methanol as a modifier</p>	<p>7.44 nmol/mg</p> <p>7.28 nmol/mg</p>	<p>Chloroform, methanol and phosphate buffer</p> <p>Supercritical CO₂ and methanol</p>	<p>Freeze dried activated sludge from domestic wastewater treatment plant</p> <p>Freeze dried activated sludge from domestic wastewater</p>	<p>Pre-treatment by vacuum freeze dryer for 24h, freeze dried sample = 0.1g; solvent system: chloroform/methanol/phosphate buffer (1:2:0.8, v/v/v); desolventization using a rotary evaporator was at < 37°C</p> <p>1 ml stainless steel extraction; extraction conditions: 80°C, 25 MPa, 15 min and 10% (v/v) methanol</p>	(Hanif et al., 2010)

1.2.2.3.2 Critical fluid extraction

1.2.2.3.2.1 Supercritical fluids

The critical point is a characteristic of a substance and it is expressed as critical pressure and critical temperature. At the critical point, defined by a *critical temperature* T_C and a *critical pressure* p_C , phase boundaries vanish. For instance, the critical point of CO₂ is 31.1°C and 7.38 MPa, and that of water is 374 °C and 22.1 MPa, above these critical points, CO₂ and water are said to be supercritical fluids (Arai et al., 2002, Sahena et al., 2009). The high density of supercritical fluids (SCF) enhances its solubilisation power and low viscosity increases its mass transfer rate (Sovova and Stateva, 2011). Compared to the conventional extraction methods, SCF requires the use of expensive pressure vessels, although, due to low operating cost, capacity to combine some operation units into one, and high product quality, the impact of this problem is minimised (Sovova and Stateva, 2011). In the recent time, supercritical fluid extraction (SFE) of lipids has gained traction as an alternative to organic solvent extraction (Garcia et al., 1996). This is partly because of the ability of SCF to selectively extract certain lipids under varied conditions of temperature, pressure and time, which allows for cost and extraction time reduction (Xu and Godber, 2000). Supercritical CO₂ and water are discussed in the next sections.

1.2.2.3.2.1.1 Supercritical CO₂

This is used in lipid extraction especially because carbon dioxide is inexpensive, non-explosive, non-flammable, non-toxic, has high purity and low critical temperature (Arai et al., 2002, Sovova and Stateva, 2011). Due to the low critical temperature, SC-CO₂ is used in the extraction of thermally labile substances in order to protect their original properties from degradation (Sovova and Stateva, 2011). Although, it dissolves more of non-polar substances, its capability to extract polar compounds can be improved by the use of co-solvents such as

methanol, ethanol and water (Sahena et al., 2009). This has been demonstrated by Hanif et al. (2010) in which they increased the yield of phospholipid fatty acids (PLFAs) from 0.5 to 7.28 nmol/mg using methanol (10%, v/v) as the co-solvent, at a temperature of 80°C, pressure of 25 MPa, 0.1g dried activated sludge and *SC-CO₂* flowrate of 2.7 mL/min. One of the factors that influences the efficiency of *SC-CO₂* is high moisture content, this must be reduced to a tolerable level, as it could hinder the rate of diffusion (Dunford and Temelli, 1997, Yamaguchi et al., 1986).

1.2.2.3.2.1.2 Supercritical water extraction

Supercritical water possesses the characteristic properties of both liquid and gases, such as diffusivity, density and heat transfer (Kritzer, 2004). These properties can be changed through the manipulation of temperature and pressure (Kritzer, 2004). At a low density, supercritical water functions like non-polar solvents and shows low dissolution for ionic substances. While at high temperature, it can dissolve organic substances, but also acts as a good solvent for gases and salts, because of a decrease in its dielectric constant, resulting from a reduction in the hydrogen bond between them and the corresponding increase in its translational and rotational motions (Kritzer, 2004, Kruse and Dinjus, 2007). Supercritical water has been studied with regards to detoxification of some less biodegradable substances, thermal gasification and hydrogen production (Clifford, 1999, Gungoren et al., 2007). Also, Gungoren et al. (2007) applied near – and supercritical water extraction in the oil recovery and products distribution from sewage sludge at temperatures of 350 – 450 °C and pressures of 21.5 – 30 MPa using solvent: sludge (3, 1.5 and 1) for 1h. The liquid yields were 42.0 – 44.4 (wt. %) and the gaseous products included hydrogen, carbon dioxide, methane and C₂ – C₄ compounds with yields of 15.3 – 20.4 (wt. %). They explained that at supercritical temperature, free radicals are produced from water, thereby favouring increasing formation of gaseous products like hydrogen (Kruse

and Gawlik, 2003). The high percentage of the gaseous products under the supercritical conditions was also attributed to cracking of larger volatile compounds (Savage, 1999).

1.2.2.3.2.2 Subcritical water extraction

Water at temperatures between its boiling point 100°C and its critical point 374°C and at pressures high enough to maintain the liquid state, exhibits a decrease in its dielectric constant, thereby mimicking the properties of organic solvents e.g methanol, ethanol (Brunner, 2009, Kruse and Dinjus, 2007, Kulkarni et al., 2011). At a lower temperatures, ionic and polar species will be extracted while at a higher temperature, particularly closer to the critical temperature, by interacting with the substrate and reducing binding forces, water readily dissolves and extracts non polar substances (Brunner, 2009). Subcritical water has been used in decontaminating soil, removing polyhydroxyalkanoates (PHA), hydrocarbons and metals in addition to extracting a range of natural products (Brunner et al., 2001, Kronholm et al., 2002). Compared to other solvents, water is said to be more attractive because it is considered a green solvent, safe, and inexpensive (Katritzky and Allin, 1996, Siskin and Katritzky, 2001). Based on these qualities of water, it can be used for extraction, hydrolysis and decomposition of organic compounds including polymeric materials (Patrick et al., 2001, Tavakoli and Yoshida, 2006).

1.2.3 Biofuels/bioenergy

Biofuel is an overarching term encompasses liquid, gas and solid fuels derived from biomass (Demirbas, 2006). Biomass itself is a term that covers material derived from terrestrial and marine plants and animals and microbes, agricultural crops/residues, organic fractions of municipal solid wastes, paper, cardboard, food waste, animal manure, green waste and other waste (Bridgwater, 1999, Demirbas, 2009, Sambo, 2000) and is regarded as potentially a third energy resource, after oil and coal (Radmanesh et al., 2006, Yaman, 2004). In addition biomass

is renewable and could be used to mitigate the effects of climate change if it were to replace the conventional fossil fuels (Radmanesh et al., 2006, Tsai et al., 2007). Therefore advantages of biofuels include in the potential to mitigate carbon dioxide emission and therefore climate change, renewable sustainable, security of supply and an opportunity to support rural economic development (Reijnders, 2006, Yan and Lin, 2009). The first and second generations of biofuels are discussed below.

1.2.3.1 First generation biofuels

First generation biofuels are produced from edible feedstock, such corn, wheat, sugarcane and sugar beet in the case of bioethanol, and palm, soybean in the case of biodiesel (Moore, 2008). The production process of the first generation biofuels is well established and large quantities have been produced worldwide (Naik et al., 2010). Although, the first generation biofuels show a net benefit in terms of greenhouse gases (GHG) emission reduction and energy balance according to many researchers, they also have the following drawbacks (Sims et al., 2008):

1. could lead to increase in the cost of food items, as a result of competition with food crops
2. potentially have a negative impact on biodiversity and may compete for scarce water resources in some regions
3. could increase the rate of deforestation
4. due to high production cost, they may not be the best option for energy security
5. could only provide limited GHG reduction benefits, although with exception of sugarcane ethanol.

1.2.3.2 Second generation biofuels

The term second generation biofuel reflects the fact the energy vectors are produced from alternative non-edible sources of biomass. For example lignocellulosic biomass which readily available and includes by-products of agriculture and forestry, such as crop residues straw, maize stover and sugar cane bagasse, and wood residues from both logging and paper industries (Abdeshahian et al., 2010, Fernando, 1994, Gomez et al., 2008, Zabaniotou et al., 2008). Current examples of second generation biofuels include cellulosic ethanol and Fischer-Tropsch fuels. These biofuels are considered to be sustainable, truly carbon neutral or even carbon negative in relation to their impact on carbon dioxide concentration, thereby mitigating the greenhouse gases (Goh et al., 2010, Naik et al., 2010). Despite numerous advantages, second generation biofuels are not cost effective because of technological bottlenecks which need to be overcome before they can potentially being used (Pauly and Keegstra, 2008, Wu et al., 2010).

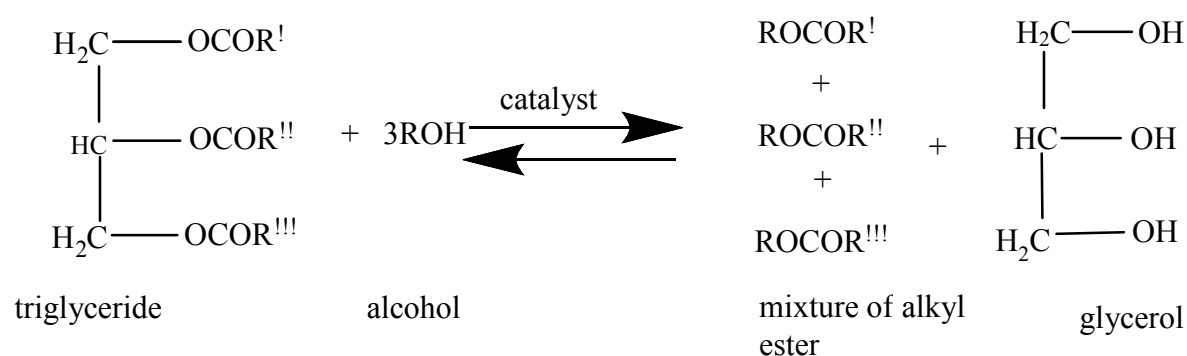
1.2.3.3 Biodiesel

Biodiesel is an alternative to fossil fuel derived diesel and contains mono-alkyl esters of long-chain fatty acids sourced from vegetable oils or animal fats (Knothe et al., 2005) and is considered to be environmentally benign as emissions contain less sulphur and aromatics while also being biodegradable (Basumatary, 2013, Deka and Basumatary, 2011, Ma and Hanna, 1999). Currently biodiesel is produced using methanol as one of its reactants, although other alcohols could be used e.g, ethanol, propanol and butanol. Methanol is preferred due to EN 14214 legislation which defines biodiesel as a methyl ester more specifically fatty acid methyl ester (FAME). Biodiesel can either be produced by transesterification reaction between triglyceride and methanol or esterification reaction involving free fatty acid and methanol in

the presence of a base/acid catalyst, or enzyme producing glycerol as a co-product (Abdeshahian et al., 2010).

As shown in Figure 1-7, in the transesterification process, triglyceride (vegetable oil) and alcohol (methanol) are reactants and reaction is catalysed by a liquid acid or base (Abdeshahian et al., 2010).

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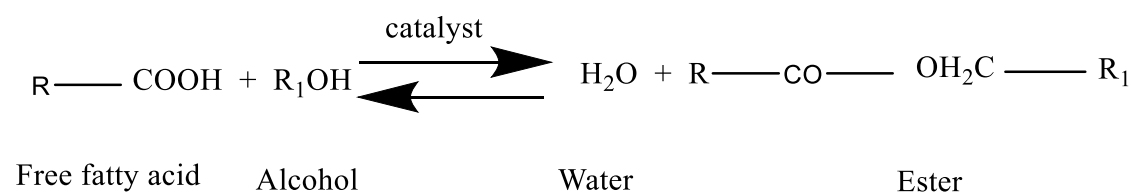


(Where R^I , R^{II} and R^{III} = carbon chain of fatty acid and R = alkyl group of the alcohol which could be methyl when methanol is used).

Figure 1-7: the transesterification of triglyceride leading to the production of biodiesel (Schuchardt et al., 1998)

The yield of methyl esters produced from the transesterification reaction depends on factors such as catalyst, alcohol-to-oil ratio, residence time, water content, amount of free fatty acid and esterifiable substances present in the sample (Siddiquee and Rohani, 2011a). The yield of methyl ester may reduce in a situation where the oil contains high amount of free fatty acids due to soap formation through saponification reaction (Abdeshahian et al., 2010). Transesterification and esterification reactions can occur simultaneously using a solid acid catalyst, leading to the formation of methyl esters in each reaction, the former involves the reaction between triglyceride and methanol while the latter involves the reaction between high

free fatty acid existing in the oil and methanol, see Figure 1-7 and Scheme 1-1 (Kulkarni et al., 2006, Meher et al., 2006).



(Where R = carbon chain of fatty acid and R₁ = alkyl group of the alcohol which could be methyl when methanol is used).

Scheme 1-1: esterification of free fatty acid (Marchetti and Errazu, 2008)

1.2.3.3.1 Production of fatty acid methyl esters (FAMES)

In the laboratory, fatty acid profile of lipids may be determined by derivatization to produce FAMES and carrying out gas chromatography (GC) analysis of the esters produced. Advantages of derivatization include: increase in the volatility of lipid constituents, better separation and reduction in the retention time (Brondz, 2002, Liu, 1994a). The production of FAMES can be driven by range of catalysts e.g. acid, base, or silylating reagents or diazomethane (Veeneman, 2011). The acid and base catalysed FAME derivatizations are the conventional method, their differences are presented in Table 1-6 (Ichihara and Fukubayashi, 2010). The three most commonly used acid catalysts used for FAME production are, hydrochloric acid (HCl), sulfuric acid (H₂SO₄) and boron tri-fluoride (BF₃) (Lall et al., 2009), HCl is the most commonly used as it has a tendency to produce quantitative FAME yield, can easily be separated from the reaction mix and it is regarded as being mild (Sheppard and Iverson, 1975). The methylating capacity of the HCl is very high while its transesterification power is low, although, this can be increased using a residence time greater than 30 min (Carvalho et al., 2004). H₂SO₄ can be used as a substitute for HCl in the methanolysis of lipids, although, it could make the polyunsaturated fatty acids to decompose at a given operating

conditions (Christie, 1989). Like HCl-methanolic reagent, BF₃-methanolic reagent has a greater methylating than transesterifying power (Liu, 1994b). BF₃-methanolic reagent has been reported to be toxic, and to have short shelf life, these properties make it less attractive and could lead to the formation of artefacts such as methyl methoxystearate and methyl methoxyoctadecenoate (Christie, 1993, Dawidowicz and Thompson, 1971, Koritala and Rohwedder, 1972). Analogous to the BF₃, is the boron trichloride (BCl₃) methanolic reagents. Although, BCl₃-methanolic-reagent is more stable at room temperature, less volatile, produces products free from contaminants, less losses of unsaturated acid than the BF₃ methylating catalyst (Brian and Gardner, 1967, Christie, 1993, Klopfenstein, 1971).

For a base-catalysed methanolysis, KOH or NaOH may be used to carry out the methanolysis reaction within 2 min at room temperature for glycerolipids while using the same catalyst might require up to 1 hr for sterol esters at 37°C (Ichihara et al., 2002, Ichihara et al., 2003).

Table 1-7: difference between base and acid catalysed methanolysis

S/N	Base catalyzed methanolysis	Acid catalyzed methanolysis	References
1.	Rapid reaction is favoured by mild temperature	Rapid reaction is favoured by high temperature (60-90°C)	(Carrapiso and Garcia, 2000, Christie, 2003, K. Liu, 1994b)
2.	Cannot catalyze the esterification of FFAs	Can catalyze the esterification of FFAs in the presence of methanol	(Ichihara and Fukubayashi, 2010, Lall et al., 2009)
3.	Less time consuming	Time consuming, up to 1-16 hr	(Lall et al., 2009)
4.	It neither degrades fatty acids nor isomerises double bond	Degrades fatty acids and isomerises double bond	(Lall et al., 2009)
5.	Might not function well in the presence of water, because of the hydrolysis of lipids	Functions well in the presence of water	(Lall et al., 2009)

(where S/N = serial number)

The choice between acidic and basic methylating catalysts depends on the moisture content and the amount of the free fatty acid in the lipid (Atabani et al., 2013, Christie, 1993, Write et al., 1944). Lipid with significant amounts of free fatty acid will require an acidic methylating

catalyst for the production of esters, otherwise there will be soap formation, see (Figure 1-8) (Kargbo, 2010). The soap thus produced can lead to the formation of a solid mass, instead of the intended methyl ester and could cause difficulty in the separation and washing of the glycerol (Kargbo, 2010).

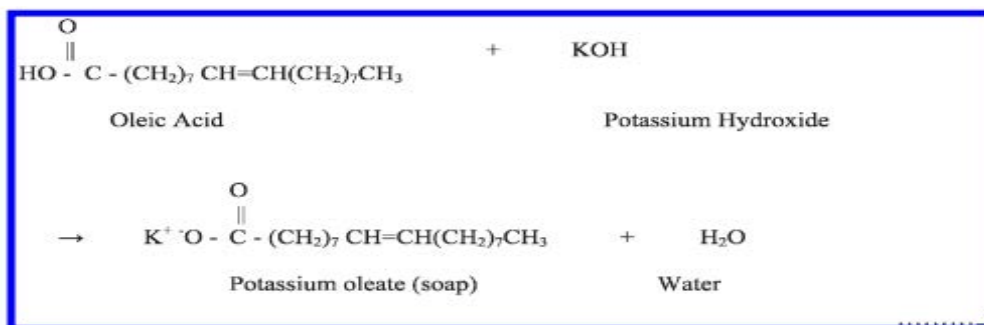


Figure 1-8: Soap formation during biodiesel production using basic catalyst (Kargbo, 2010).

1.2.3.3.2 Biodiesel (FAMES) production from activated sludge

Works carried out by some researchers on the production of FAMES (biodiesel) from the lipid extracted from sludge at the laboratory scale, are hereby presented.

Huynh et al. (2012) studied the possibility of producing fatty acid methyl ester (FAME) from wet activated sludge using subcritical water – subcritical methanol (SCW – SCM). Their objective was to assess the potential of using subcritical water for hydrolysis and as a catalyst during FAMES production. At the optimum operating conditions of 175°C, methanol-to-sludge ratio 30 mL/g, 5g of water, 1g of activated sludge (84 wt. % H₂O) and pressure 3.5 MPa, they obtained the highest FAME yield of 45.58 ± 3.52 wt.%, after 24 h. The predominant FAMES were those from myristic acid (5.00 ± 0.09 area %), palmitic acid (71.53 ± 0.64 area %), stearic acid (11.30 ± 0.30 area %), oleic acid (3.32 ± 0.19 area %) and elaidic acid (4.59 ± 0.22 area %). They also carried out in situ acid catalysed methanolysis on dry sludge at 55°C, using 25

mL/g of methanol-to-sludge ratio, 1g of activated sludge and 4% H₂SO₄, and obtained a FAME yield of 58.99 ± 1.34 wt.% after 24 hr. The predominant FAMES were those from myristic acid (5.85 ± 0.05 area %), palmitic acid (73.28 ± 0.48 area %), stearic acid (10.18 ± 0.31 area %), oleic acid (2.88 ± 0.02 area %) and elaidic acid (4.16 ± 0.05 area %). They concluded that FAMES production using SCW-SCM is possible without a distortion to the profile while subcritical water (SCW) could be used as an alternative to the acid catalyst.

Mondala et al. (2009) worked on the production of biodiesel from the municipal primary and secondary sludge (activated sludge) by in situ transesterification reaction. They investigated the effects of temperature, acid concentration and methanol-to-sludge ratio on the yield of FAMES. The highest yield of FAMES 14.5% and 2.5% for the primary and activated sludge, respectively, were obtained at the optimum operating conditions 75°C, 5% (v/v) H₂SO₄, residence time 8 h and methanol-to-sludge ratio 12:1. They found that the yield of the FAMES obtained from the secondary sludge was independent of the interactive effects of the factors investigated, unlike that from the primary sludge. The composition of the FAMES showed that the prominent fatty acids in the sludge were palmitic acid (C16:0), stearic acid (C18:0) and oleic acid (C18:1). They also estimated the cost of FAMES production to be \$3.23/gallon which is lower than those of petroleum diesel and soybean biodiesel and concluded that sludge from municipal sewage works is a great potential feedstock for producing biodiesel due to economic and abundance reasons.

Revellame et al. (2011) investigated the production of biodiesel from wet activated sludge (water content of 84.5%) by in situ transesterification reaction and obtained the highest FAMES yield to be 3.93 ± 0.15 % at the optimum conditions of 75°C, methanol-to-sludge ratio 30 mL/g, catalyst (sulfuric acid) concentration 10% (v/v) and residence time 24 h. They carried out gas chromatography (GC) analysis on the product recovered from the reaction and found that according to order of magnitude that the predominant methyl esters were palmitic acid (C16:0),

oleic acid (C18:1), palmitoleic acid (C16:1), linoleic acid (C18:2) and stearic acid (C18:0). While in terms of appearance from GC, were palmitic acid, palmitoleic acid, stearic acid, oleic acid and linoleic acid and suggested that this order was an indication that the method used for converting the lipid content of the activated sludge to biodiesel was suitable for the process. They also carried out an economic analysis on the biodiesel obtained from wet activated sludge (water content of 84.5%) and dried activated sludge (water content of 5%) by in situ transesterification. Their results showed that it was more costly to produce biodiesel from the wet than from the dried activated sludge. Thus, they concluded that a yield of at least 10 wt. % must be obtained in order for biodiesel produced from activated sludge to be competitive favourably.

Revellame et al. (2010) produced biodiesel through in situ transesterification of activated sludge using 4 x 6 x 5 factorial design representing temperature, methanol-to-sludge ratio and the concentration of catalyst (sulfuric acid), respectively, to optimise the process. They obtained optimum conditions: temperature 55°C, methanol-to-sludge ratio 25 mL/g and sulfuric acid concentration 4% (v/v) which gave a biodiesel yield of $4.79 \pm 0.02\%$ after 24h reaction. The results obtained from analysing the product recovered after the transesterification indicated the predominance of methyl esters of palmitic acid, palmitoleic acid, stearic acid and linoleic acid and this shows that activated sludge is a potential feedstock in biodiesel production.

Dufreche et al. (2007) worked on the production of biodiesel by in situ transesterification of dried secondary sludge using 200 mg of ground sample with 5% moisture content, temperature 50°C, 1 mL 1% sulfuric acid in methanol and obtained a biodiesel yield of 6.23%, after an overnight reaction. They also worked on the methanolysis of lipid extract obtained from solvent extraction of secondary sludge using lipid extract of 20mg, 1% sulfuric acid in methanol 2 mL and temperature 50°C. They obtained a highest yield of biodiesel 4.4wt.% after an overnight

reaction from the lipid extracted using a combination of n-hexane, methanol and acetone. The results of the biodiesel analysis revealed that the profile of five prominent FAMES was in the following order of magnitude palmitic acid (C16:0), oleic acid (C18:1), stearic acid (C18:0), palmitoleic acid (C16:1) and linoleic acid (C18:2). They concluded that biodiesel production by in situ transesterification resulted to the highest yield compared to the methanolysis of the lipids extracted from activated sludge.

Siddiquee and Rohani (2011a) investigated the production of biodiesel through the methanolysis of lipids extracted from the primary and secondary sludges using hexane and methanol, respectively. They used the modified Christie's method involving temperature 60°C, 20 mL of 2% H₂SO₄, 200 mg of lipid extract dissolved in 10 mL of hexane, and residence time 24h. The yields of the FAMES produced from the lipids extracted from primary sludge using hexane and methanol were 41.25 (wt/wt) % and 38.94 (wt/wt) %, respectively while those obtained from the secondary sludge were 26.89 (wt/wt) % and 30.28 (wt/wt) %, respectively. The results obtained also showed that the natural zeolite used as a dehydrating agent increased the biodiesel yield by approximately 18 (wt/wt) %. The composition of the FAMES showed that the palmitic acid methyl ester (C16:0) was the most prominent compared to the methyl esters of myristic acid (C14:0), palmitoleic acid (C16:1), oleic acid (C18:1) and linoleic acid (C18:2). They concluded that the mixture of primary and secondary sludges is good for biodiesel production and lipid extraction is better done using methanol, since it is a reactant in the transesterification process.

Pastore et al. (2013) investigated the in situ methanolysis of the primary and secondary sludges under the following conditions: 10g of dried sludge, 100 mL of methanol, 0.25 mL of 96% H₂SO₄, temperature of 65°C and reaction time of 7 h. They obtained FAMES yield of 10.0 wt. % and 1.9 wt.% from the primary and secondary sludges, respectively. They also worked on the extraction of lipid from dewatered primary sludge and then converting it by methanolysis

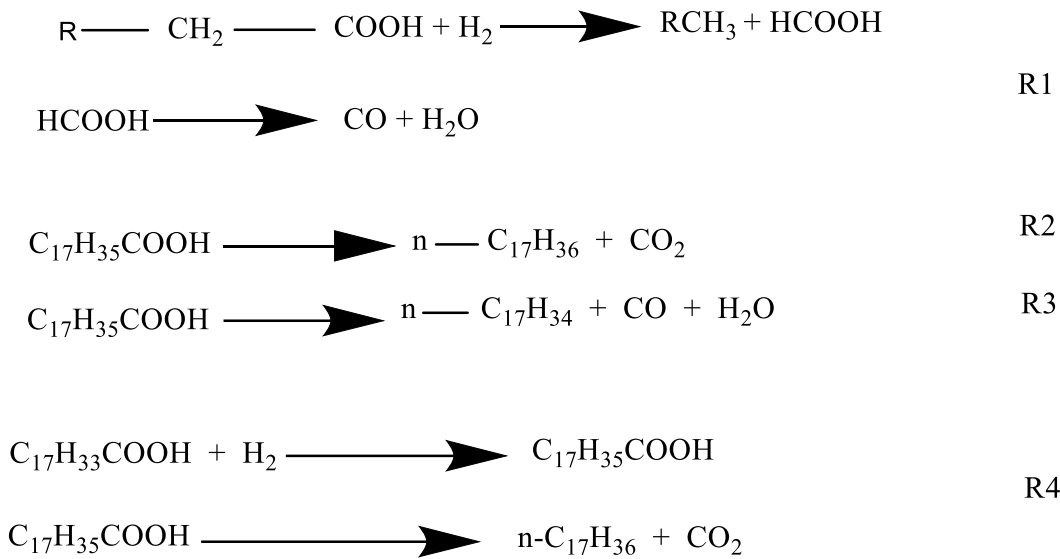
to FAMES. This was carried out by stirring 150 g of dewatered sludge (with total suspended solid of 14.4%) and 300 mL of hexane at a temperature of 311 K for 3 h, after which the resulting lipid product was converted to FAMES by mixing 20 mL of methanol with 0.20 mL of sulfuric acid at a temperature of 343 K for 2 h. They obtained a lipid and FAMES yield of 2.9 ± 0.1 g (13.4 wt. %) and 2.0 ± 0.1 g (9.0 wt. %), respectively. However, these lipid and FAMES yields increased to 4.6 ± 0.2 g (21.1 wt. %) and 3.9 ± 0.2 g (17.9 wt. %) when 2 mL of sulfuric acid (96%) was added to the dewatered primary sludge before lipid extraction and they concluded that this method gave the optimum yield of biodiesel using a less amount of energy and at a reduced cost.

Zhu et al. (2014) compared the yields of the biodiesel produced from the lipid extracted from municipal sludge using acid hydrolysis, Soxhlet apparatus and water bath shaking extraction methods. The biodiesel was produced by acid-catalysed methanolysis at 75° C, 10 mL of 5% (v/v) H₂SO₄, 10 g dry sludge, 8 h residence time, 12: 1 methanol-to-dry ratio and 50 mL hexane (for better solubility of the reaction matrix). The results obtained showed that the yields of the biodiesel from the lipids extracted using acid hydrolysis, soxhlet apparatus and water bath shaking were 1.30, 6.35 and 4.10%, respectively. The composition of the biodiesel was independent of the method used in extracting the lipid and the most predominant methyl esters were palmitic, stearic, elaidic and myristic acids. They concluded that biodiesel produced from the lipid extracted using Soxhlet extraction gave a higher yield.

1.2.3.4 Renewable diesel

Renewable diesel is a petrodiesel-like fuel derived from biological sources (Knothe, 2010) and it has the following advantages over biodiesel, better quality fuel, adaptability with the existing refinery processes facilities (Kalnes et al 2009). It can be produced from catalytic deoxygenation of fats and oils via hydrotreating of triacylglycerides or free fatty acids (Do et al.,

2009, Kalnes et al., 2007, Snare et al., 2006). Hydrotreatment is carried out using a high partial pressure H_2 gas and catalysts such as $CoMo/Al_2O_3$ and $NiMo/Al_2O_3$ (Lestari et al., 2009, Smith et al., 2009). The hydrogen gas helps to remove the oxygen atoms in the fatty acids in form of water molecule (Fu et al., 2011a). The deoxygenation of fatty acid to hydrocarbon can be achieved through decarboxylation and decarbonylation, see Scheme 1-2 (Idesh et al., 2013). Decarbonylation reaction removes oxygen atom as carbon monoxide and water, producing olefinic hydrocarbon (Snare et al., 2006). This type of reaction, can also occur during hydrotreating. For instance, carboxylic acid can undergo hydrogenolysis reaction to produce hydrocarbon and formic acid, with the latter product decomposed to carbon monoxide and water (see R1) (Boda et al, 2010). Decarboxylation involves the removal of carboxyl group and therefore the oxygen atom in the fatty acids resulting to paraffinic hydrocarbon, without the need of hydrogen gas (Fu et al., 2011a, Snare et al., 2006). The hydrocarbon molecule produced has a high energy density compared to that obtained through hydrotreating process (Fu et al., 2011a). Hydrogenation reaction might occur prior to decarboxylation, depending on the degree of saturation of the fatty acid, as seen in the production of hydrocarbons from oleic acid (R4).

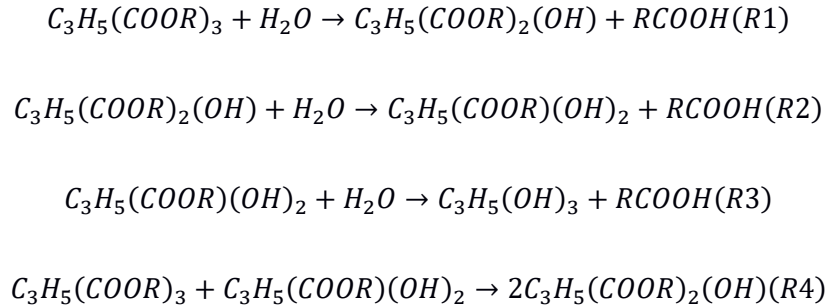


Where $\text{C}_{17}\text{H}_{35}\text{COOH}$ = stearic acid, $\text{n} - \text{C}_{17}\text{H}_{36}$ = heptadecane, $\text{n} - \text{C}_{17}\text{H}_{34}$ = heptadecene and oleic acid $\text{C}_{17}\text{H}_{33}\text{COOH}$

Scheme 1-2: hydrogenolysis of carboxylic and decomposition of formic acid (R1), acid decarboxylation (R2), decarbonylation (R3) of stearic acid to hydrocarbon and hydrogenation (R4) (Wang et al., 2012, Boda et al, 2010)

Apart from the direct methods of producing hydrocarbon fuel through hydrotreating, decarboxylation and decarbonylation, renewable diesel can be derived from the hydrolysis of triacylglycerides (TAG) to free fatty acid (FFA) using subcritical water, before subjecting the fatty acids to decarboxylation (Wang et al., 2012). The process describes by Wang et al involves the conversion of TAG to diglyceride (DAG) and FFA, and then to monoglyceride (MAG) and FFA and finally to FFA and glycerol, see Figure 1-9. Thus, three moles of subcritical water and one mole of triglyceride are required to produce three moles of FFA and one mole of glycerol. The free fatty acids produced may either be separated from the

hydrolysate before decarboxylation or the hydrolysate is used directly in the production of the hydrocarbon fuels (Li et al., 2010, Wang et al., 2012).



Where TAG = $C_3H_5(COOR)_3$; DAG = $C_3H_5(COOR)_2(OH)$; MAG = $C_3H_5(COOR)(OH)_2$
FFA = and Glycerol = $C_3H_5(OH)_3$

Figure 1-9: hydrolysis mechanism of triglycerides leading to the production of free fatty acid (Moquin and Temelli, 2008)

Researchers have carried out some investigations on the deoxygenation of free fatty acids to hydrocarbons in either organic solvents or water medium, using catalysts, at near- and supercritical conditions. Although, none of the works used lipid extract from activated sludge as the feedstock, a review of some of these investigations are hereby presented.

Rozmyslowicz et al. (2010) investigated the potential of producing biofuel by deoxygenating a by-product from pulp industry: tall oil fatty acid (TOFA) using dodecane (solvent) and palladium on carbon catalyst. The deoxygenation was carried out at temperatures between 300 and 350°C, pressure 17 bar, and varied amount of hydrogen, TOFA concentration ranging from 0.15 to 0.6 mol/L and TOFA pre-hydrogenation temperature 120°C. The pre-hydrogenation of TOFA was done in order to suppress the by-products of the reaction which could affect the yield of the desired hydrocarbons. The results obtained showed that deoxygenation of TOFA

was dependent on temperature and catalyst loading. At the optimum conditions of 350 °C, 330 min and TOFA concentration of 0.15 mol/L, the selectivity of heptadecane and conversion of TOFA were 72 % and 32 %, respectively. The addition of hydrogen ensured that the activity of the catalyst was maintained during the reaction. It was also observed that in near-absence of hydrogen, the reaction favoured the production of stearic acid. They concluded that deoxygenating TOFA to hydrocarbon, especially C₁₇ in the presence of catalyst, was achievable.

Watanabe et al. (2006) studied the decarboxylation of stearic acid in supercritical water (SCW) using alkali hydroxides (KOH and NaOH) and metal oxides: CeO₂, Y₂O₃ and ZrO₂ catalysts. The decarboxylation was conducted using 0.3 g of stearic acid, 0.3 g of (CeO₂, Y₂O₃ and ZrO₂), 1.0g of water (0.167 g/cm³) under the supercritical conditions of 673 K and 25 MPa, and argon pressurized at 1 MPa for 30 min. The same experiment was repeated using 1.0 M KOH and NaOH in place of water. They reported without the influence of argon, that under supercritical conditions, a conversion of 2% of stearic acid was achieved resulting to CO₂ and C₁₆ alkenes as the main products, but, this was increased to 13 and 32 % using KOH and NaOH, respectively, with the formation of CO₂ and heptadecane. While the use of CeO₂, Y₂O₃ and ZrO₂ metal catalyst favoured the decarboxylation of stearic acid to CO₂ and pentadecene with the conversions of 30, 62 and 68%, respectively. In the presence of argon and without supercritical water, they recorded a stearic acid conversion of 50% favouring CO₂ and heptadecane, as the main products. They concluded that catalysts supported the decarboxylation of stearic acid.

Fu et al. (2010) reported that higher yields of pentadecane could be obtained from the hydrothermal decarboxylation of palmitic acid using 5% palladium on activated carbon (Pd/C) and 5% platinum on activated carbon (Pt/C) compared to metal salts (NaCl, MnCl₂, CoCl₂, CuSO₄ and MgSO₄) and bases (NaOH and KOH) investigated. They demonstrated that Pt/C

and Pd/C were more effective in producing hydrocarbon through hydrothermal decarboxylation from fatty acid, although, with the latter catalyst showing greater catalytic effect. The yield of pentadecane was 63% obtained at temperature 370°C, residence time 3h using 20 mg of 5% Pd/C. While 76% yield was obtained at temperature 370°C, residence time 1h using 15 mg of 5% Pt/C. These results are contrary to those obtained by Snare et al. (2006), as they have demonstrated that 5% Pd/C (1 g) gave more than 98% conversion of stearic acid to C₁₇ products at 300 °C and pressure of 600 kPa using 86 g of dodecane and 4.5 g of stearic acid (0.154 mol/L) for 6 h. Although, Fu et al. (2010) argued that this difference may have resulted from the suspected influence of the solvent (dodecane) used by Snare et al. (2006) in conducting the deoxygenation on the catalyst activity. Fu et al. (2010) also reported that a temperature of 290°C, batch holding time of 6 h, palmitic acid loading of 50 mg and Pt/C loading of 5 mg were required to obtain a near completion of deoxygenation reaction in experiments carried out at 290, 310, 330, 350 and 380°C for 2, 4, 6, 8 and 10 h, respectively. Their results further revealed that selectivity was increased at a lower temperature than at higher temperature, probably due to the formation of more by-products emanating from possible cracking at higher temperature. To investigate the impact of carbon chain and degree of unsaturation on the products of hydrothermal decarboxylation of fatty acid, Fu et al. (2011a) carried out experiments using 0.176 mmol of saturated (stearic, palmitic and lauric acids) and unsaturated (oleic and linoleic acids) fatty acids, and 5 mg Pt/C at 330°C with water occupying 95% of the reactor volume (0.167 mL) under the reaction conditions. They reported that the products (n-alkanes) of the decarboxylation of saturated fatty acids were independent of the carbon number and gave high selectivity of more than 90 %. While the unsaturated fatty acids showed low selectivity with the formation of stearic acid which was followed by the decarboxylation reaction to produce heptadecane. They suggested that since the hydrothermal decarboxylation of unsaturated fatty acid resulted to hydrogenation to stearic acid, that there

was possibility of in situ production of hydrogen from either water or fatty acid molecules, under the reaction conditions considered (Fu et al., 2011b). Due to high cost of Pt/C and Pd/C, Fu et al. (2011b) carried out experiments to investigate whether activated carbons can be used for the hydrothermal decarboxylation of palmitic and oleic acids (fatty acids) to hydrocarbons. They loaded a 1.67 cm³ batch reactor with 0.195 mmol fatty acids, 0.25 – 0.72 cm³ of water and 15 mg of carbon and conducted the hydrothermal reaction at 370°C for 3h. They reported conversions of palmitic acid of 20 – 33% with molar yield of 9-19% and selectivity of about 38 – 58% to pentadecane. The conversion of oleic acid to hydrocarbon was 80 ± 4% with a heptadecane molar yield of 6 ± 1% and selectivity of 7 ± 1%. They suggested that the low yield of heptadecane may have risen from the gasification of a small amount of oleic acid and the production of stearic acid might be due to hydrogenation reaction with hydrogen produced from the possible reaction of water and carbon catalyst and also from water gas shift reaction. According to them, the presence of products C₁₂ – C₁₆ and C₉ – C₁₃ fatty acids was attributed to C - C bond cracking reaction.

1.2.4 Design of Experiment

Design of experiment (DoE) or experimental design is a statistical technique used to set up a series of experiments that combine multiple variables/parameters and allows for the simultaneous evaluation of these experiments to obtain a measurable response (Bezerra et al., 2008, Dejaegher and Heyden, 2011, Hibbert, 2012, Telford, 2007). Therefore DOE is used for optimising process parameters, analysing and interpreting data (Hibbert, 2012). When using DoE to optimise process parameters, it is not always possible to obtain a good shape which depicts a maximum of a function, although, it is necessary to get responses which meet the minimum criteria (Hibbert, 2012). In order to increase the chances of getting a good maximum or minimum response, quadratic terms are required (Hibbert, 2012).

Design of Experiment has various types and they include factorial, Plackett-Burman, central composite, Box-Behnken, Doehlert, D-optimal and mixture designs and are differentiated by the model which can be obtained from them (Hibbert, 2012). For instance, factorial design gives rise to a linear or first order model and Box-Behnken, central composite and Doehlert designs result to a quadratic model (Bezerra et al., 2008). Central composite design and the various divisions of the design of experiment are described below. The descriptions of other types of DoE are not considered due to restriction on the number of words required in this thesis. The choice of the type of DoE depends on the factors or variables (number and usage) and preference of the experimenter (Dejaegher and Heyden, 2011). The design of experiment is divided basically into three, which are screening, response surface and mixture designs (Dejaegher and Heyden, 2011). It can be carried out according to the flowchart shown in Figure 1-10

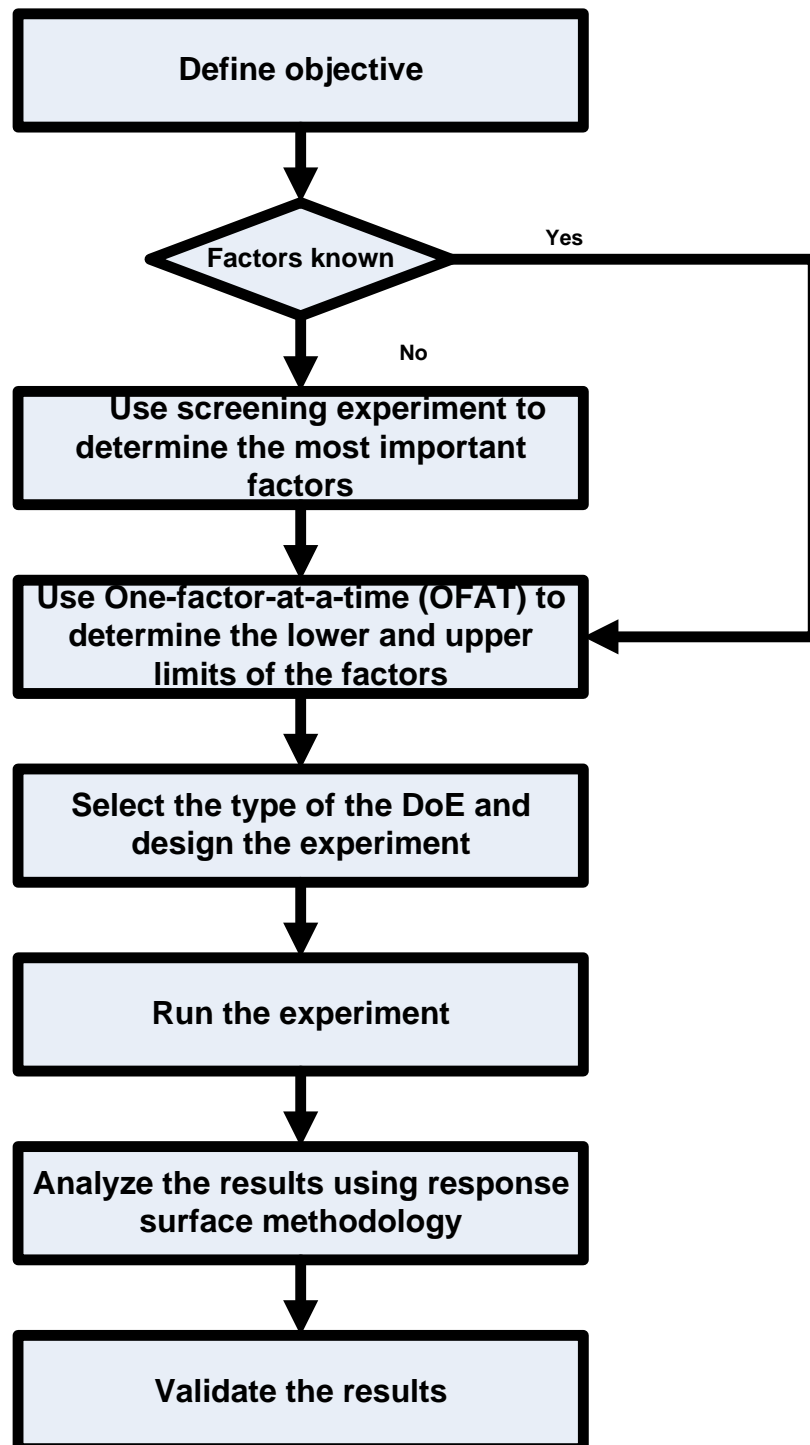


Figure 1-10: a flowchart for Design of Experiment

1.2.4.1 Central composite design (CCD)

CCD is the most commonly used DoE, as it allows more than two level designs, identification of the interaction between factors, potential of assessing the experimental region completely

and opportunity of carrying out less number of experiments (Hibbert, 2012, Tixier et al., 2011). It comprises two-level factorial or fractional factorial design, axial or star point and centre point, see Figure 1-11. The number of experiment (N) to be performed using CCD is usually represented by Equation 1-1 (Bezerra et al., 2008, Ferreira et al., 2007).

$$N = 2^f + 2f + N_c$$

Equation 1-1

Where 2^f , $2f$ and N_c are two-level factorial design, star point and number of replicates at the center points, respectively and f represents number of factors.

The factorial and axial designs determine the interaction and quadratic terms, respectively (Myers et al., 1995).

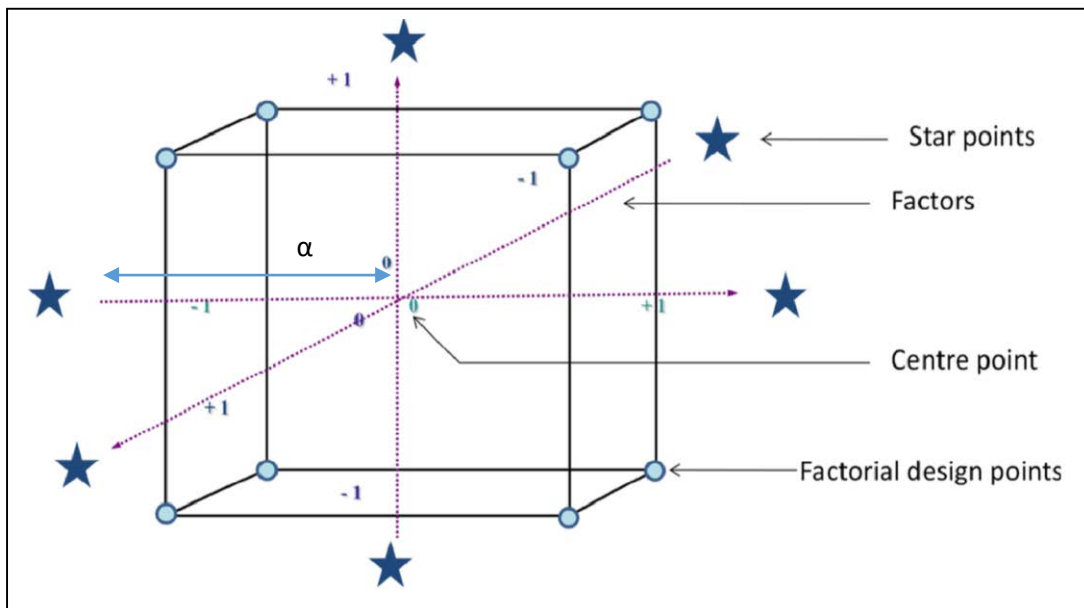


Figure 1-11: Central composite design (Tixier et al., 2011)

The star points are located at a distance of α to the centre, they provide opportunity for estimating experimental error and curvature for the model (Anderson and Whitcomb, 2005, Tixier et al., 2011). The α values are between 1 and \sqrt{k} , representing face centered and spherical designs, where k is the number of factors (Anderson and Whitcomb, 2005). In face centred

design (FCCCD), the star points lie at the centre of the factorial space while in the spherical design, they are located on the circumference of the circle (Figure 1-12) (Ferreira et al., 2007, Hibbert, 2012). The advantages of FCCCD include less number of factors (three factors) and less expensive and is usually being considered when there is an equality between regions of interest and that of operation (Anderson and Whitcomb, 2005, Ferreira et al., 2007).

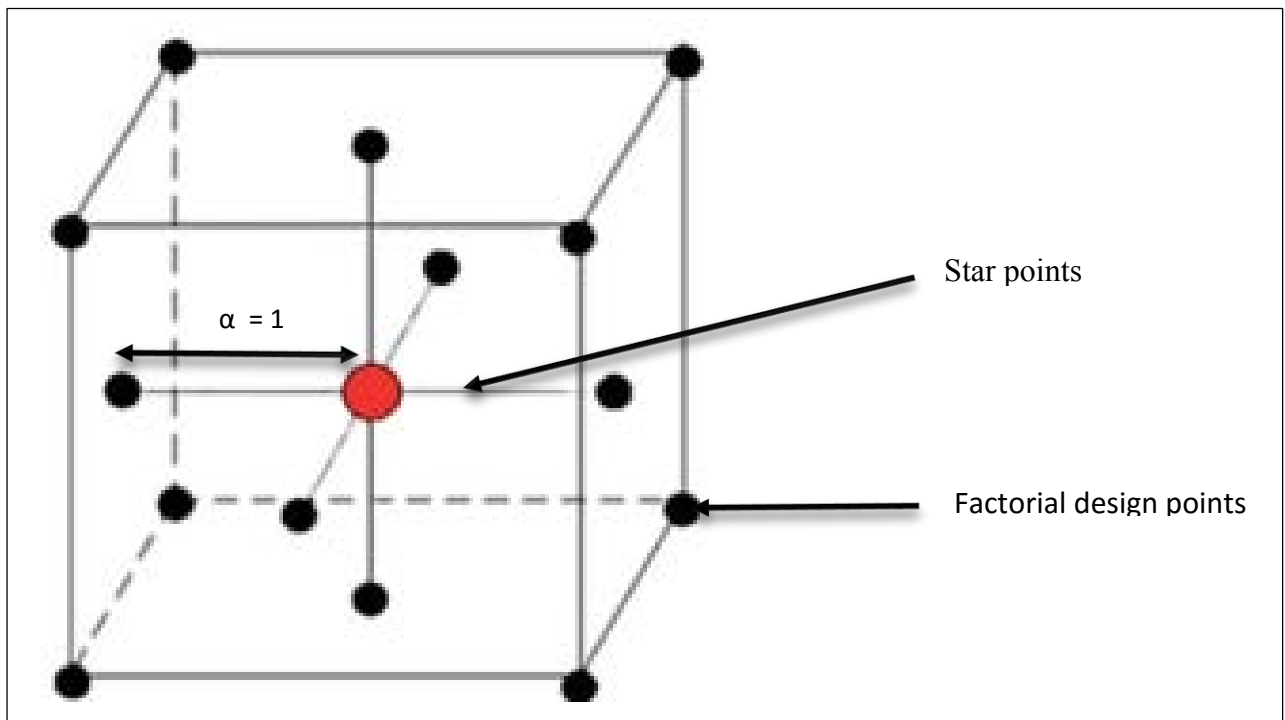


Figure 1-12: face centred central composite design (Lebed et al., 2014)

1.2.4.2 Screening experiment

Screening experiment is used to identify the most prominent factors which have a significant effect on a given response (Bezerra et al., 2008, Dejaegher and Heyden, 2011). It allows factor evaluation at 2-level and it include full factorial, fractional factorial or placket-Burman designs (Dejaegher and Heyden, 2011). In the literature, 2-level factorial design was used by Siddiquee and Rohani (2011a) to investigate the effects of solvent to sludge ratio, temperature and

extraction time on the lipid extraction from primary and secondary sludge. They used methanol and hexane for the lipid extraction. The lipid yields obtained using methanol were 14.46 and 10.04 (wt/wt) % from primary and secondary sludge respectively. While the lipid yields obtained using hexane were 11.16 and 3.04 (wt/wt) % from primary and secondary sludge, respectively. They also found that temperature was the most significant factor in the design model, followed by solvent to sludge ratio when using methanol to extract lipid from both primary and secondary sludge.

1.2.4.3 Response surface methodology

Response surface methodology is a set of statistical and mathematical techniques used to develop, analyse and optimize processes which extends DOE (Mjos and Waktola, 2015). RSM is aimed at understanding the interaction of process parameters of a given experiment to obtain an optimum results (Bezerra et al., 2008, Dejaegher and Heyden, 2011). The advantage of RSM over optimizing techniques such as Simplex method is that it provides useful information about the experiments to be carried out before it is done (Hibbert, 2012). The models such as linear or quadratic obtained from RSM are used to describe the system (Bezerra et al., 2008, Skartland et al., 2011). The general model obtained from the response surface design for f factors is given in Equation 1- 2 (Dejaegher and Heyden, 2011)

$$y = \beta_0 + \sum_{i=1}^f \beta_i x_i + \sum_{i \leq j}^f \beta_{ij} x_i x_j + \sum_{i=1}^f \beta_{ii} x_i^2 \quad \text{Equation 1-2}$$

Where y = response, β_0 = intercept, β_i = main coefficients, β_{ij} = interaction effect, β_{ii} = quadratic coefficients, $(x_i x_j)$ = interaction terms

The models describe the relationship between the response and factors. The fitness of the models to experimental data can be evaluated using ANOVA, residual analysis and external

validation (Dejaegher and Heyden, 2011). For a model to fit experimental data, it must be significant and its lack of fit should be insignificant (Bezerra et al., 2008).

1.2.5 Objectives

In the light of the introduction and literature review, the main objective of this project was to study the utility of subcritical water to support the extraction of lipids from activated sludge, and downstream production of biodiesel and renewable diesel. To achieve this, the objective is further divided as enumerated below:

- (1). Investigate the utility of subcritical water in lipid extraction from activated sludge (Chapter 4)
- (2). Optimize the process variables involved in the subcritical water mediated lipid extraction from activated sludge in order to ensure maximum recovery (Chapter 4)
- (3). Develop an understanding of the operating parameters required to support catalytic methanolysis of lipids derived from activated sludge to produce fatty acid methyl esters (FAMEs) (Chapter 3)
- (4). Assess the potential of producing renewable diesel from the lipids derived from activated sludge through catalytic hydrothermal decarboxylation (Chapter 5)

2 CHAPTER 2 MATERIALS AND METHODS

2.1 INTRODUCTION

This Chapter presents the materials and methods used throughout the project targeted at a critical evaluation of the utility of subcritical water to support the production of biodiesel and renewable diesel from the lipid fraction obtained from activated sludge. It covers the sources of the materials, their applications and how they were used in achieving the objectives of each segment of the project. These involve, the materials and methods used in recovering solid activated sludge from its slurry, extracting lipids from the biomass and water, producing fatty acid methyl esters (FAMES) and renewable diesel. The materials and methods used with the instrumentations: high performance thin layer chromatography (HPTLC), titrimetry and gas chromatography (GC), in analysing lipids and products derived from them, are presented.

2.2 CHARACTERIZATION OF THE ACTIVATED SLUDGE

Samples

Activated sludge slurry was collected from Finham and Minworth Sewage Treatment Works (STW), United Kingdom, respectively, at different seasons, as shown in Table 2-1.

Table 2-1: collection of activated sludge from the industry

S/N	STW	Date supplied	Batch	Weight (Kg)
1	Finham	04/07/13	1	5.127
2	Finham	20/08/13	2	21.433
3	Finham	17/12/14	3	22.548
4	Minworth	04/09/14	1	11.556
5	Minworth	28/11/14	2	19.392

(STW = sewage treatment works, Finham and Minworth STWs are located at Coventry and Birmingham, both in United Kingdom and S/N = serial number)

(a). Finham Sewage Treatment Works

Finham STW is owned and managed by Severn Trent Water Ltd. It is located in the southern part of Coventry, United Kingdom and serves over 450, 000 people (Figure 2-1). It receives an average of 120 Ml/d of sewage through Sowe and Sherbourne inlets (Severn Trent Water, 2015). The sewage is from predominantly domestic and industrial sources with small amount of agricultural wastes (Watstech, 2015).

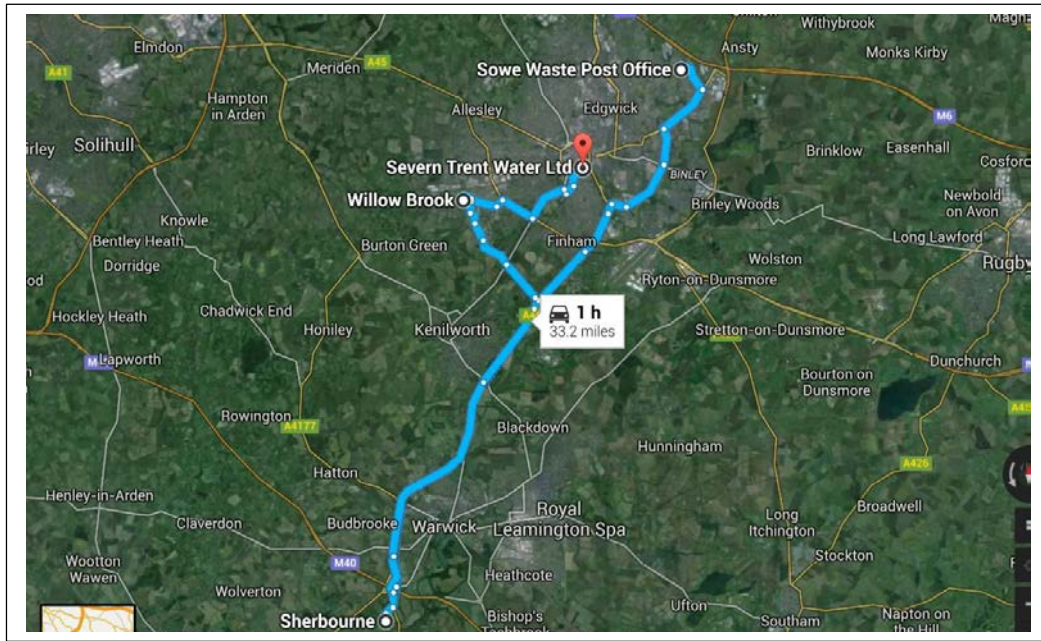


Figure 2-1: a Map showing Severn Trent Water Ltd where the Finham Sewage Treatment Works is situated, Coventry, United Kingdom (Map data ©2016 Google)

(b). Minworth Sewage Treatment Works

This sewage works like Finham STW, is a subsidiary of Severn Trent Water Ltd. It treats waste from domestic and industrial sources, equivalent of 1.7 million people (Severn Trent Water, 2015). It covers Walsall (North), Dudley (West) and Sutton Coldfield (South) with an area of approximately 400 Km² (Figure 2-2). This STW receives sewage from smaller works and has an average daily supply of 5.8 m³/s (Severn Trent Water, 2015).

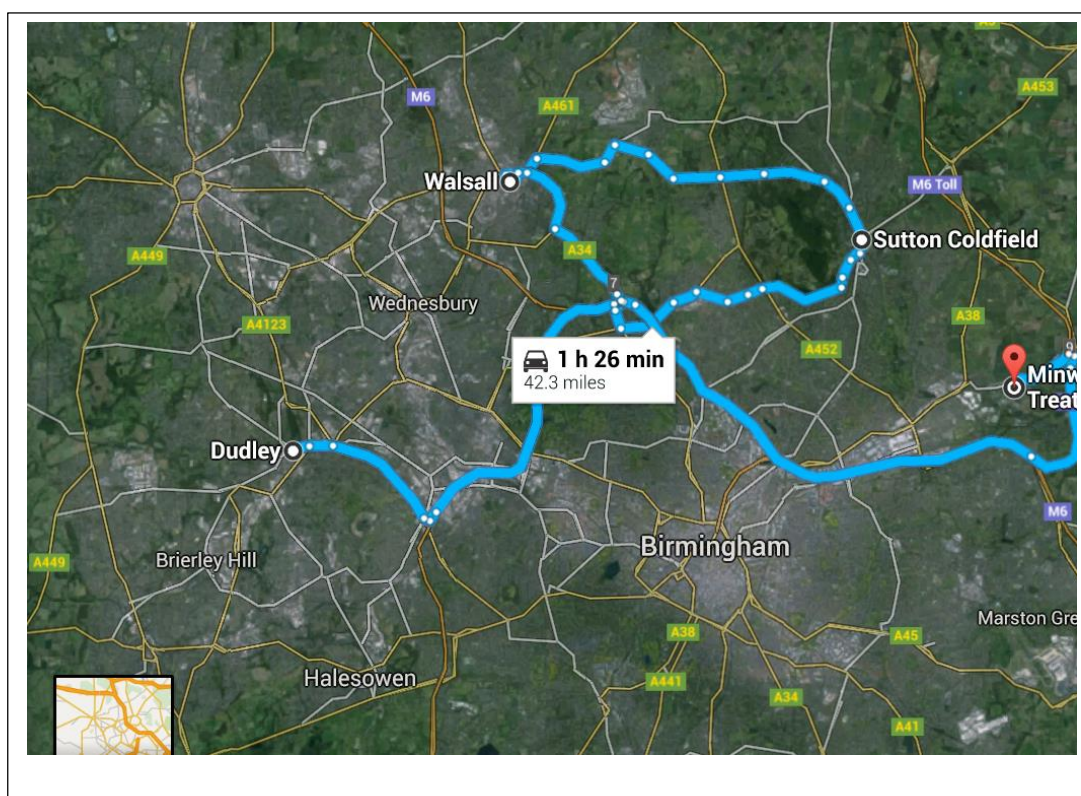


Figure 2-2: a Map showing Minworth Sewage Treatment Works, West midlands, UK (Map data ©2016 Google)

(2). Equipment / apparatuses

Re-circulating filtration fume cupboard (Astecair 5000E, carbon filter type AC/AMN BLEND, a Willaire Scientific Company) was used to ensure health and safety. Memmert oven (Atmosafe) was used to supply the required temperature for drying the activated sludge. Stainless Steel round tray (30.0 cm diameter) was used as a container for activated sludge drying. GSW-15K (Good Scale) was used for determining the weight of activated sludge slurry. Mettler Toledo (AB204-S) was used for determining the weight of the dried activated sludge. Desiccator was used for cooling the activated sludge to ensure that there was no moisture gain. Filter paper (pore size of 11 μm and diameter of 185 mm, Fisher brand) was used with vacuum filtration apparatuses for filtering the activated sludge slurry.

2.2.1.1 Experimental procedure for characterising activated sludge

The activated sludge slurry was weighed before it was filtered using a vacuum filtration apparatus. Aliquot of it was put in the filter holder containing a filter paper with a pore size of 11 μm and the vacuum pump was switched on to initiate filtration. As the filtration continued, the volume of the activated sludge slurry reduced and hence, more of it was added. This process continued, until the entire activated sludge slurry had been filtered. The resulting liquid and dewatered biomass were used to determine the total suspended solid (TSS) and total dissolved solid (TDS). The flowchart of the process is illustrated in Figure 2-3.

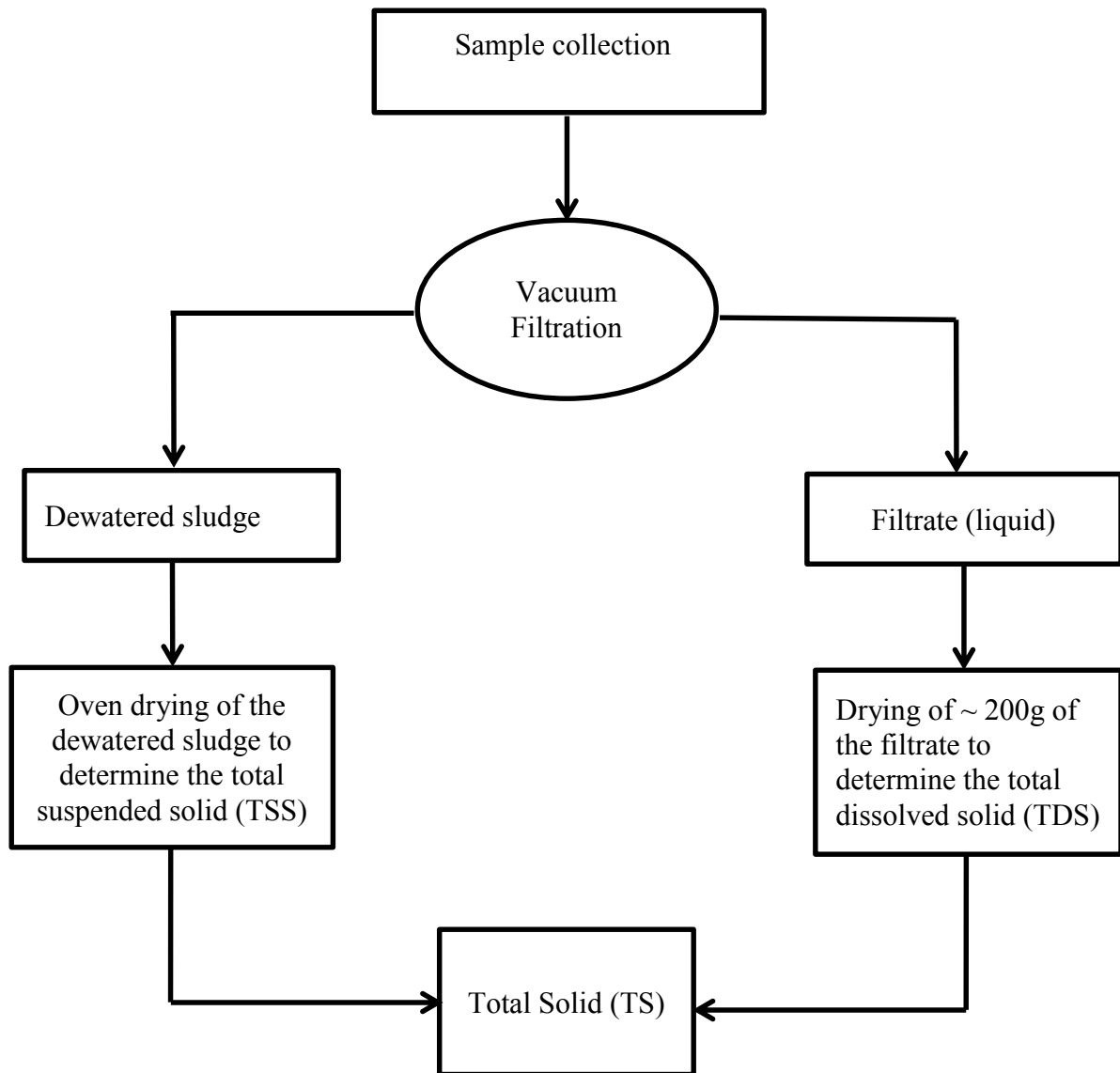


Figure 2-3: Flow chart representing the procedure used in the activated sludge biomass recovery

2.2.1.1.1 Biomass partitioning within the activated sludge

The dewatered activated sludge biomass was used to obtain the TSS by drying at 50°C until constant weight was reached and using Equation 2-1 to calculate its value. While the liquid obtained post filtration of the activated slurry was used to recover the TDS. This was done by determining the total weight of the liquid, before 200 mL from it was dried at 50 °C to a constant weight and extrapolating to obtain the TDS, using Equation 2-2. The total solid (TS) was obtained by the summation of TSS and TDS (Equation 2-3). The moisture content of TS was determined using the method described in Section (2.2.1.1.2). The TSS, TDS and TS were measured in grams.

$$TSS = X - Z \quad \text{Equation 2-1}$$

$$DS = X - Z$$

$$TDS = DS \times w \quad \text{Equation 2-2}$$

$$TS = TSS + TDS \quad \text{Equation 2-3}$$

Where, X = weight of the drying container plus dried activated sludge; Z = weight of the empty container, TDS = total dissolved solid; DS = dissolved solid; w = total weight of the filtrate

2.2.1.1.2 Determination of the percentage moisture content of the activated sludge

The activated sludge used in this experiment was obtained from the total solid (TS) (Section 2.2.1.1.1). In order to determine the percentage moisture content of the total solid, 1g of the activated sludge (TS) was put in the oven and dried at 105 °C for 24 h. The dried biomass was put in the desiccator and allowed to cool down to the ambient temperature. The percentage moisture content (%MC) was determined using Equations 2-4, after the weight of the dried biomass was obtained.

$$\%MC = \frac{(x - xf)}{x} \times 100 \quad \text{Equation 2-4}$$

Where xf = actual weight of the dried activated sludge and x = initial weight of the activated sludge

2.3 LIPID EXTRACTION FROM ACTIVATED SLUDGE USING ORGANIC SOLVENT

This was performed according to the Soxhlet and Folch methods, described below.

(1). Samples

These were the activated sludge biomass from Finham and Minworth STWs (batch 1)

(2). Materials

Chloroform (reagent plus, 99.8, Sigma-Aldrich) and methanol (99+%, Fisher Scientific) were used as organic solvents for extracting lipid, and sodium chloride (99.999%, Fisher Scientific) for removing water from the organic layer.

(3). Equipment/apparatuses

Re-circulating filtration fume cupboard (Astecair 5000E, SN: 0322, carbon filter type AC/AMN BLEND, a Willaire Scientific Company) was used to ensure health and safety. Vacuum filtration apparatuses (glass vacuum filter holder, Edwards high vacuum pump, KnF D-79112-Freiburg vacuum pump and PYREX vacuum filter flask) was used for separating activated sludge-solvent mixture. Barnstead Lab-Line 2052-1 Mult-Blok heater (Thermo Scientific) was used for drying the organic layer containing lipid extract. LABCOLD Sparkfree Labfreezer (Labcold, Medical & Scientific Refrigeration) was used for preserving the lipid extract. Mettler-Toledo (AB204-S, Mettler-Toledo International Inc.) was used for measuring the weight of activated sludge, lipid extract and sodium chloride. Separating funnel (conical, 50 and 250 mL, Sigma-Aldrich) was used for separating organic and aqueous phases. Soxhlet apparatuses (condenser, extractor, distillation flask and heating mantle) were used for extracting lipid. Mortar and Pestles (1 L mortar and Pestle porcelain 215mm length, Fisher Scientific) was used for grinding the activated sludge into smaller particles. Screw top vial (4

mL, 15 x 45 mm, amber with white closed to cap, PTFE/Si Septa, 5183-4450 and 5183-4301, Agilent Technologies LDA UK Limited) was used for storing lipid. Extraction thimble (cellulose, single thickness, 22mm x 70mm, 25 thimbles, GE Healthcare, Life Sciences, Whatman) was used for holding the activated sludge during extraction. Filter paper (pore size of 11µm and diameter of 185 mm, Fisher Scientific UK) was used for filtering the activated sludge-solvent mixture.

2.3.1.1 Experimental procedure of lipids extraction using Soxhlet apparatus

A weight of 5g of dried activated sludge biomass was weighed into the thimble and 175 mL of methanol was measured into a 250 mL distillation flask containing 5 boiling chips. This volume of methanol corresponded to 70% of the distillation flask capacity. The chips were used to ensure that methanol boiled gently and that bumping was prevented. The Soxhlet apparatus was set up in the fume cupboard, by putting the distillation flask in the heating mantle, connecting the extractor which enclosed the thimble and then fixing the condenser. The bottom and top outlets of the condenser were connected to the water source and vent, respectively. The water source was turned on and the methanol in the flask was heated to 65°C. As the methanol boiled, its vapour condensed and the liquid formed, tickle down into the thimble to extract lipid from the activated sludge. This process continued until the volume of methanol reached a certain height in the siphon and then flowed down to the distillation flask. This cycle continued until 30 runs were completed, see Table 2-2. The process was repeated using 35 and 40 runs. Each run was not repeated due to insufficient amount of activated sludge biomass.

Table 2-2: Conditions for the extraction of lipids from activated sludge using Soxhlet apparatuses

S/N	Temperature (°C)	weight of the sample (g)	Number of runs	Volume of the solvent (mL)
1	65	5	30	175
2	65	5	35	175
3	65	5	40	175

(where S/N = serial number)

At the end of extraction, the volume of lipid extract solution was reduced using a rotary evaporator at 50°C and further dried to a constant weight by passing a stream of nitrogen gas into it at a temperature of 50°C. The rotary evaporator and nitrogen gas were used to ensure that methanol evaporated at a lower temperature than its boiling point of 64.6°C, so as to prevent thermal decomposition of lipids. The lipid yield was obtained gravimetrically using Equation 2-5. Afterwards, the recovered lipid extract was stored in the freezer at -20°C, prior to analysis (Figure 2-4).

Equation 2-5

$$\% \text{ lipid yield} = \frac{\text{weight of the lipid extract}}{\text{weight of the dried activated sludge (total solid)}} \times 100$$

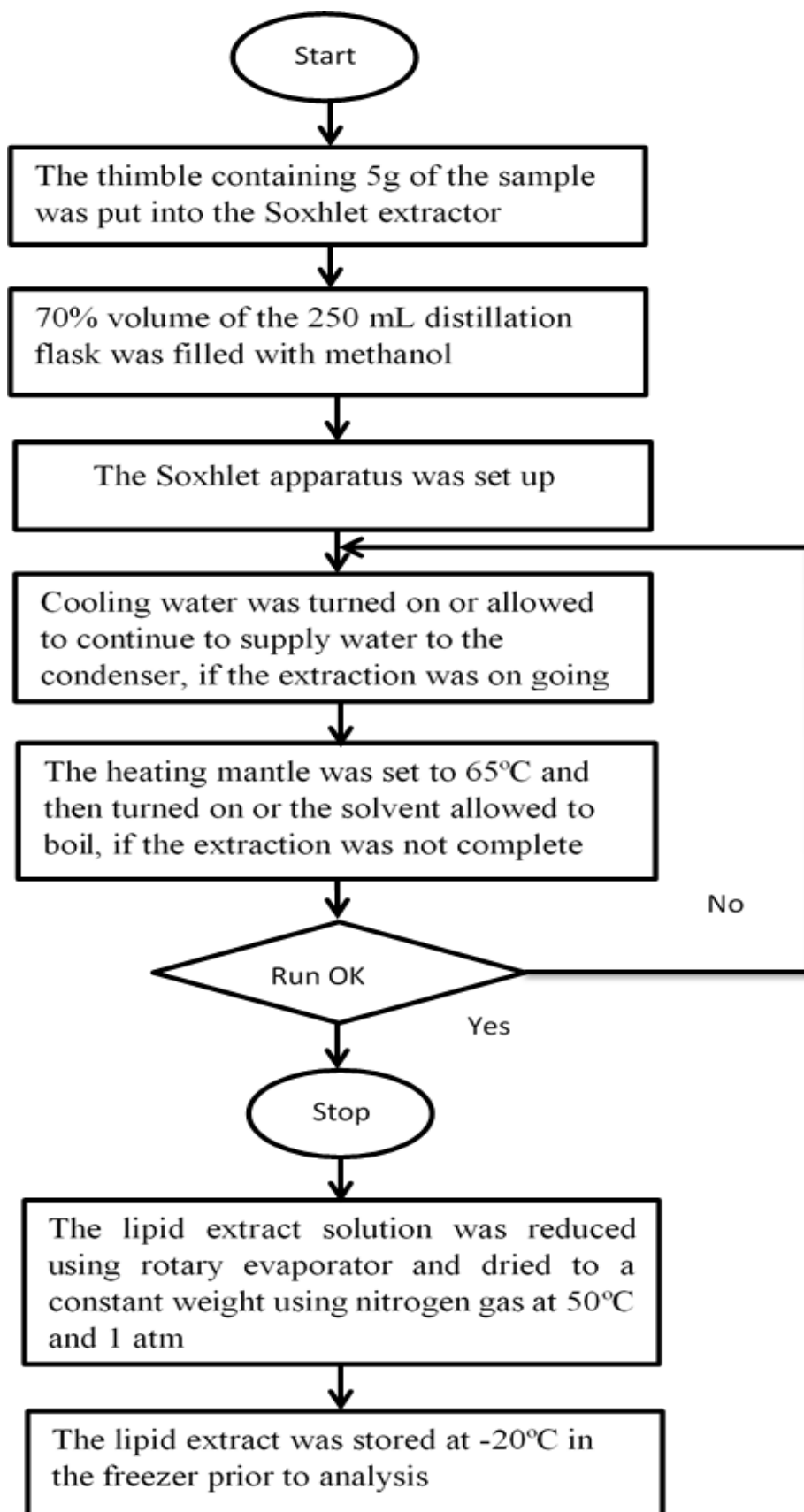


Figure 2-4: a flow chart illustrating the procedure involving the use of Soxhlet method in extracting lipid from activated sludge

2.3.1.2 Lipid extraction from activated sludge using Folch method

The Folch method combines chloroform and methanol in extracting lipids from biological materials, followed by washing of the extract matrix with water/salt and/or water/salt/methanol, in order to separate the non-lipids substances at ambient condition (Folch et al., 1957).

2.3.1.2.1 Experimental procedure for extracting lipid from activated sludge biomass using Folch method

A weight of 1g of dried activated sludge biomass was ground using mortar and pestle, before, it was put in a 50 mL Erlenmeyer flask and homogenized with 20 mL of chloroform/methanol (2/1, v/v), before the flask was stoppered. The flask was vortexed for a period of 20 min at 200 rpm and room temperature using orbital shaker. The resulting mixture was then filtered using vacuum filtration apparatus containing filter paper with pore size of 11 μ m. The filtrate containing the desired lipids was put in a 50 mL conical separating funnel and washed with 4 mL of 0.9% w/v of sodium chloride in water solution. Thereafter, it was vortexed vigorously for 30s and then clamped unto a retort stand, before its content was allowed to settle. When 5 min had elapsed, it was observed that there were two distinct layers of liquid in the funnel. The lower (organic) layer containing the lipids was drained into a 50 mL distillation flask. The upper (aqueous) layer was washed further with 4 mL of 0.9 (wt/v) % sodium chloride and then with 2 mL of a mixture of methanol and 0.9% w/v sodium chloride (1/1, v/v), in order to recover all the lipids in the aqueous layer. The volume of lipid-solvent obtained was reduced using a rotary evaporator at 50°C and dried to a constant weight by passing it through a stream of nitrogen gas at 50°C and atmospheric pressure.

In order to obtain high amount of lipid for the production of FAMES and renewable diesel, the Folch method was scaled up by using 5g, 100 mL of chloroform/methanol (2/1, v/v), 20 mL of 0.9 sodium chloride in water (wt /v) % and 10 mL of a mixture of methanol and 0.9% sodium chloride in water (wt/v) (1/1, v/v) while following the procedure described above. The activated sludge – solvent mixture was vortexed for 100 min and 250 mL Erlenmeyer and distillation flasks were used in place of 50 mL employed with 1g activated sludge biomass. The entire process of extracting lipids from activated sludge has been summarized in Figure 2-5 below.

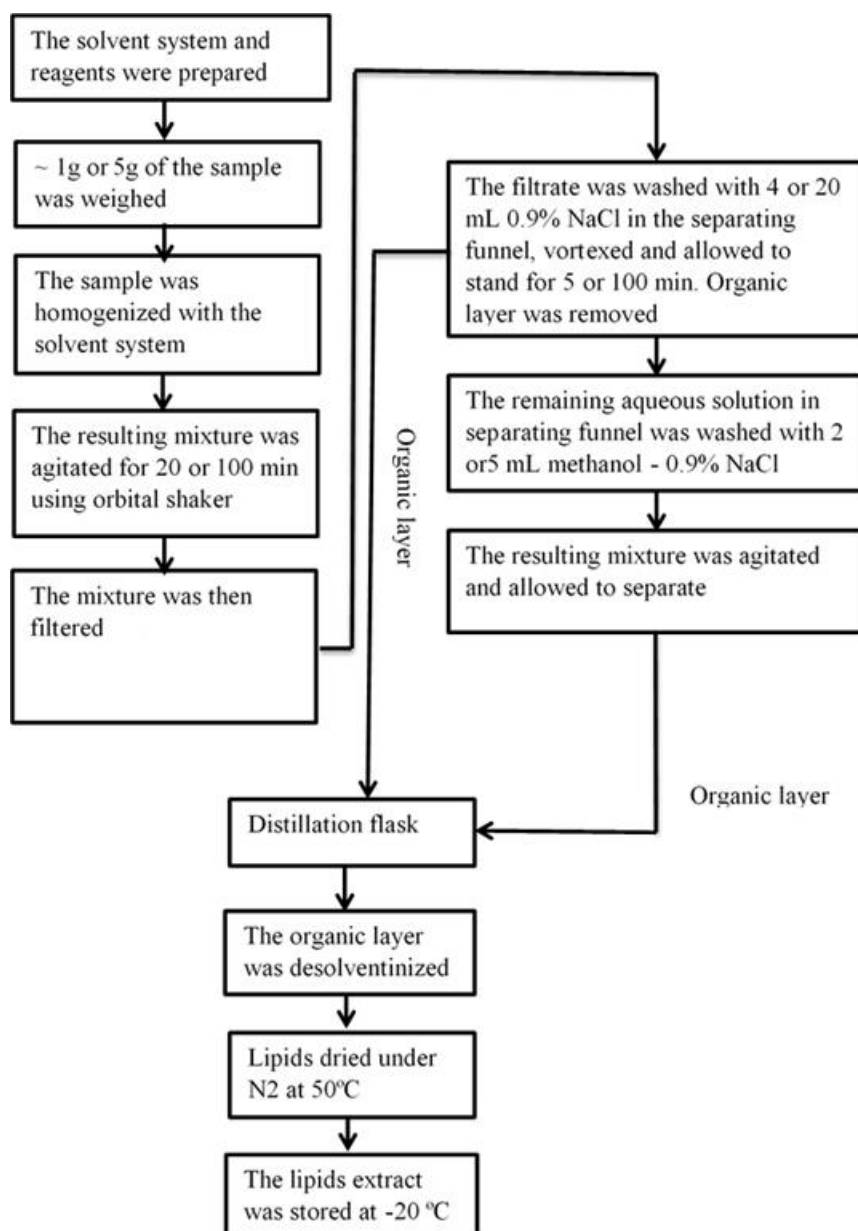


Figure 2-5: a flow chart illustrating the procedures involved in extracting lipid from activated sludge using Folch method

2.4 PRODUCTION OF FATTY ACID METHYL ESTER FAMES

2.4.1 Sample

This was the lipid extract obtained from the activated sludge (Finham STW, batch 2) using the Folch method.

2.4.2 Materials

Nitrogen gas (99%+, supplied by the British Oxygen Company (BOC)) was used to ensure that the organic layer was dried at a low temperature. Sodium chloride (99.999%, Fisher Scientific) was used to remove trace amount of water in the organic layer. Boron trichloride in methanol (12% w/w, SUPELCO) and hydrogen chloride in methanol solution (~1.25M HCL, for GC derivatization, Fluka Analytical) were used as methylating reagents and catalysts. The 2, 2-Dimethoxypropane (reagent grade, 98%, Sigma-Aldrich) was used as water scavenger. Grain FAME Mix, 10mg/mL in CH₂Cl₂ (SUPELCO) was used as FAMES standard. Heptadecanoic acid (>98%, Sigma-Aldrich) was used as internal standard. The lipid standards: erucic acid (C₂₂H₄₂O₂, 99%, capillary GC), nervonic acid (Cis-15-Tetracosenoic acid, 99+%, capillary GC), arachidonic acid (5, 8, 11, 14-eicosatetraenic acid, 99%, capillary GC), palmitoleic acid (cis-9-Hexadecenoic acid, 99%, capillary GC), gamma-linolenic acid (6,9,12-Octadecatrienoic), Cis-8,11,14-Eicosatrienoic acid (Homo-gamma-linolenic acid, ~99%), myristoleic acid (Cis-9-Tetradecenoic acid, 99%, capillary GC), petroselinic acid (cis-6-Octadecenoic acid, 99%, capillary GC) and stearic acid were purchased from Sigma-Aldrich. Palmitic acid was bought from Cayman Chemical Company and oleic acid (99%) from Alfa Aesar.

2.4.3 Equipment

The experiment was performed in a re-circulating filtration fume cupboard (Astecair 5000E, SN: 0322 carbon filter type AC/AMN BLEND, a Willaire Scientific Company) to ensure health and safety. Jouan C 422 centrifuge machine (Labx media group) was used to separate organic and aqueous layers. Barnstead Lab-Line 2052-1, Mult-Blok Heater (Thermo Scientific) was used to generate the required reaction temperature and for drying the organic layer. Sartorius Research Electronic Semi-Microbalance (Analytical products Ltd, England) was used for

measuring the weight of organic materials. 5 mL micro reaction vessel was used for carrying out the methanolysis reaction.

2.4.4 Experimental procedure

FAMES were produced from lipids (lipid standards and extract) by using a method adapted from Supelco (2014). The amount of the lipid and heptadecanoic acid (internal standard) put inside the reaction vessel were 5 mg and 200 μ g, respectively. Followed by 3 mL of 1.25M hydrochloric acid in methanol, before vortexing. To the resulting mixture was added 100 μ L of 2, 2-dimethoxy propane. The methanolysis reaction was then initiated at a constant temperature of 85 °C and allowed for varied residence times 0.083, 0.25, 0.5, 1.0, 12, 24 and 72h, respectively. At the end of which the reaction was quenched in a pool of ice, before 1 mL of hexane and 1 mL of 0.9% aqueous NaCl were added. The resulting mixture was put in a 25 mL centrifuge tube and centrifuged at 4000 RPM (2236 g) for 10 min. Thereafter, Pasteur pipette was used to aspirate the organic layer containing the FAME into a 4 mL pre-weighed amber vial while the spent catalyst and glycerol with the trapped FAME remained in the centrifuge tube. The recovered FAME was then dried in a stream of nitrogen gas at 50°C and 1 atm, and weighed. The process of aspirating the FAMES, drying and weighing continued, until a constant weight was obtained. The experiment was repeated using 12% w/w boron trichloride in methanol at a residence time of 60 min.

The FAMES of the lipid standards were also produced using the above experimental procedure with 1.25M hydrochloric acid in methanol at a constant residence time of 30 min. The resulting products were analysed using GC-FID (Section 2.9.3.1), and the reaction was monitored using CAMAG HPTLC (Section 2.9.2.2).

2.5 PALMITIC ACID AND VEGETABLE OIL RECOVERY FROM WATER

2.5.1 Materials and equipment

The organic solvents used were hexane (99% pure, mixture of isomer, purchased from ACROS Organics), diethyl ether for residual analysis (Sigma-Aldrich) and toluene (analytical reagent, purchased from Fisher Scientific UK). The free fatty acid used was palmitic acid (purchased from Cayman Chemical Company) while the vegetable oil was Solesta Sunflower oil (bought from ALDI). Pipette M1000 (fully adjustable, positive displacement, 10 μ L to 100 μ L, purchased from Fisher Scientific UK) was used to transfer palmitic acid and vegetable oil solutions, water and organic solvent into the 15 mL centrifuge tube. Nitrogen gas (99.99%, British Oxygen Company, UK) was used with Barnstead La-Line 2052-1 Mult-Blok Heater (Thermo Scientific) for drying the extract. Jouan C 422 Centrifuge Machine (Labx Media Group) was used to separate the organic and aqueous phases from the lipid mixture. Sartorius Research Electronic Semi-Microbalance (Analytical products Ltd, England) was used for weighing of materials. 230 mm disposable glass Pasteur pipettes (Volac) was used to aspirate the organic phase from the palmitic acid-water or vegetable oil-water mixture. Pipette M100 (fully adjustable, positive displacement, 10 μ L to 100 μ L, purchased from Fisher Scientific UK) was used in preparing the concentrations of the palmitic acid and vegetable oil. Rotamixer (Hook & Tucker Instrument, England) was used in mixing the palmitic acid-water or vegetable oil-water mixture before centrifuging. 4 mL amber screw top vial (purchased from Agilent Technologies LDA UK Limited) was used as a container for drying the organic product and for storing the resulting palmitic acid and vegetable oil.

2.5.2 Experimental procedures

2.5.2.1 Palmitic acid recovery from water

A concentration of 5 mg/mL of palmitic acid in hexane, diethyl ether and toluene, respectively, were prepared in a 2 mL amber Eppendorf tube and transferred to three different 15 mL centrifuge tubes. Followed by addition of 1 mL of distilled water and 1 mL of hexane, diethyl ether and toluene into their respective palmitic acid solutions. The resulting mixture was thoroughly vortexed and centrifuged at 1500 rpm (302 g) for 5 min. The organic phase was aspirated using the Pasteur pipette into three different pre weighed 4 mL amber vials, differentiated by the solvent type and dried at 50 °C under streams of nitrogen gas at 1 atm, before it was weighed. The process of aspirating the organic phase, drying and weighing continued. Each time, the result was compared with the previous one, until a constant weight was obtained. The experiment was conducted in triplicate.

2.5.2.2 Vegetable oil recovery from water

This was carried out as presented in Section 2.5.2.1, using vegetable oil in place of palmitic acid. 5 mg of vegetable oil solutions in hexane, diethyl ether and toluene, respectively were used.

2.6 SUBCRITICAL WATER MEDIATED LIPID EXTRACTION FROM ACTIVATED SLUDGE

2.6.1 Preliminary experiment for subcritical water mediated lipid extraction

2.6.1.1 Sample

This was a ground dried activated sludge biomass from Finham STW (batch 2) with a moisture content of 11.84 %.

2.6.1.2 Materials and Equipment

Distilled water was used as a solvent for the lipid extraction. Hexane (99% pure, mixture of isomer, purchased from ACROS Organics) was used to recover the lipids post subcritical water extraction. Hewlett 5890 Packard Series II oven (Hp Hewlett Packard) was used to supply the required temperature. The temperature inside the reactor was monitored using thermocouple (Type k). R 160 P Sartorius Research Electronic Semi-Microbalance (Analytical products Ltd, England) was used to measure the weight of sample. 8in Open End and Ring Spanner Wrench, 10"-250 mm Heavy-Duty-Drop-Forged and 200mm-8 inch (SupaTool) Adjustable Wrenches were used to assemble the reactors and fit one of them to the thermocouple. The reactor fittings were purchased from Swagelok.

The reaction vessels were three batch reactors constructed using 316 stainless steel pipe (purchased from Swagelok). Two of the reactors were capped at both ends using 316 SS cap for $\frac{3}{4}$ in. The remaining reactor was capped at one end using 316 SS cap for $\frac{3}{4}$ and at the other fitted with a reducer $\frac{3}{4}$ to $\frac{1}{4}$ in where the thermocouple (type k) was connected. The dimensions of the reactors were volume (22.5 mL), length (114mm), internal diameter of the reactor (15.45 mm), external diameter of the reactor (19.09 mm) and wall thickness (1.67 mm), see Figure 2-6.



Figure 2-6: mini batch reactor connected with a thermocouple inside Hewlett 5890 Packard Series II plus GC oven (1 = thermocouple, 2 = mini batch reactor and 3 = floor of the oven)

2.6.1.3 Experimental procedures

Three mini batch reactors were charged with 0.7g ground activated sludge biomass and 15 mL distilled water. The caps of the reactors were fastened, before they were vortexed manually. To the reactor with a reducer, a thermocouple adaptor was inserted into it, until the tip was half way, see Figure 2-6. The thermocouple was connected to a digital readout where the temperature reading of the reaction inside the reactor was obtained. The three reactors were put in the GC oven, before it was switched on and set above the desired temperature. The temperature of the oven was reduced when that of the reactor was close to the set point. At the end of the extraction, the reactors were cooled to the room temperature in a bucket of ice water. The three reactors were decoupled and the extracts were collected in 50 mL centrifuge tubes. The experiments were carried out in triplicate varying temperature at 80 and 140°C while the biomass loading, time and water remained constant at 0.7g, 47 min and 15 mL, respectively. An example of the temperature profiles obtained is presented in Figure 2-7. The lipids were

recovered using a modified procedure presented in Section 2.5.2. Hexane was used to recover lipid from the products of the extraction, since it gave a better result than diethyl ether and toluene in Section 2.5.2.1. The volume of hexane that was added to the products of the extraction was 4 mL and no amount of water was included. The 50 mL tubes were centrifuged at 4000 rpm (2236 g) for 30 min and the supernatant was decanted.

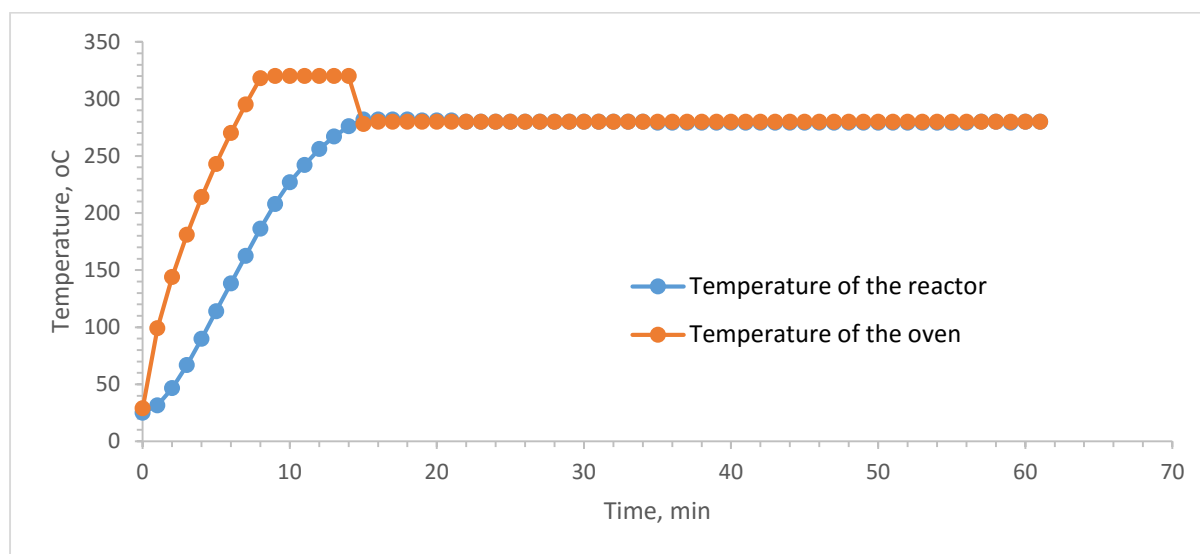


Figure 2-7: an example of the temperature profiles obtained from the subcritical water extraction of lipids from activated sludge at 280°C for 47 min

2.6.2 One-factor-at-a-time (OFAT) experiment for the subcritical water lipid extraction from activated sludge

This was done according to the experimental procedure presented in Section 2.6.1.3 and was carried out in triplicate, by varying one parameter across the roll of Table 2-3 and keeping others constant along the column while the volume of water remained unchanged. For instance, when biomass loading was varied (1, 5 and 8%), temperature and time were kept constant at 80°C and 20 min, respectively, while the volume of water was maintained at 15 mL.

Table 2-3: conditions for OFAT experiment on subcritical water mediated extraction of lipid from activated sludge

S/N	Factors	Conditions		
		A	B	C
1	Biomass loading, %	1.0	5.0	8.0
2	Temperature, °C	80	140	280
3	Time, min	20	40	80
4	Water loading, mL	15	15	15

2.6.3 Design of experiment for the subcritical water mediated lipid extraction from activated sludge using face centred central composite design (FCCCD)

2.6.3.1 Sample: dried activated sludge from Minworth STW (batch 1)

The upper and lower values of the factors: temperature (80 and 280°C), time (20 and 80 min) and biomass loading (1 and 8%) were used in Design-Expert 7.0.0 software to design the experiment using FCCCD. This involved one replicate of factorial point, four centre points in each factorial block, one replicate of axial (star) point and two centre points in each axial block

The conditions (Table 2-4) obtained were employed in conducting the experiments according to the procedure presented in Section 2.6.1.3.

Table 2-4: experimental conditions for face centred central composite response surface design (FCCCD) for subcritical water mediated extraction of lipid from the activated sludge

Run	Block	Factor 1	Factor 2	Factor 3
		A: Temperature(°C)	B: Time (min)	C: Biomass loading (%)
1	Day 1	280	20	1.0
2	Day 1	180	50	4.5
3	Day 1	280	80	8.0
4	Day 1	180	50	4.5
5	Day 1	80	80	1.0
6	Day 1	280	80	1.0
7	Day 1	180	50	4.5
8	Day 1	80	20	1.0
9	Day 1	80	20	8.0
10	Day 1	180	50	4.5
11	Day 1	280	20	8.0
12	Day 1	80	80	8.0
13	Day 2	180	80	4.5
14	Day 2	180	50	8.0
15	Day 2	180	50	4.5
16	Day 2	180	50	1.0
17	Day 2	80	50	4.5
18	Day 2	280	50	4.5
19	Day 2	180	20	4.5
20	Day 2	180	50	4.5

At the end of the extraction, the product from each run was filtered using filter paper (pore size of 11 μm , diameter of 185mm, Fisherbrand) placed in a glass vacuum filter holder (Sartorius Stedim, Biotech) connected to high vacuum pump (Edwards). The lipid in the supernatant (liquid phase) was recovered using the modified procedure presented in Section 2.5.2.1, with hexane as the organic solvent. The activated sludge biomass was recovered as the solid after filtration. This was further dried at 50°C in the oven (Memmert, AtmosSAFE) over night and weight measured using Mettler-Toledo, analytical balance (AB204-S, Mettler-Toledo International Inc). The amount of the biomass solubilised was calculated by the difference in the initial and final weights of the activated sludge pre and post subcritical water mediated lipid extraction. The percentage biomass solubilised was determined by the ratio of the amount solubilised to the initial weight of the activated sludge fed into the reactor multiplied by 100. Lipid was extracted from the recovered activated sludge using Folch method, see Section 2.3.1.2.1.

2.6.3.1 Validating the results of the design of experiment from the subcritical water mediated lipid extraction

The lower and upper lipid yields (7.1 and 41%) obtained from Section 2.6.3 were used to generate operating conditions in Design-Expert 7.0.0 Software which could be used to carry out experiments in order to validate the results predicted using FCCCD. Out of the twenty conditions generated, temperature 280°C, residence time 30 min and biomass loading 1% were used to perform the experiment, because they showed a desirability of 0.912. The total lipid yield and biomass solubilised were obtained using the method presented in Section 2.6.3. The experiments were conducted in triplicate. The sample used was activated sludge biomass (total solid) from Finham STW, as that from Minworth STW was exhausted. We expect a disparity in the results of the lipid yield and biomass solubilisation due to difference in the sources of activated sludge.

2.7 PRODUCTION OF RENEWABLE DIESEL

2.7.1 Samples

Lipid extract was obtained from the activated sludge (Minworth STW, batches 1 and 2) using the Folch method. Palmitic acid (99%) and oleic acid (99%) were purchased from Cayman Chemical Company and Sigma-Aldrich, respectively. They were used as model free fatty acids to produce hydrocarbon products: pentadecane and heptadecane which were used to assess the efficacy of the method developed for hydrothermal decarboxylation of lipid extract from activated sludge.

2.7.2 Materials

Distilled water was used for carrying out the hydrothermal decarboxylation reaction. Platinum on activated carbon (5wt % Pt/C) and palladium on activated carbon (5wt % Pd/C) were purchased from Sigma-Aldrich and they were used to catalyse the hydrothermal decarboxylation reaction.

2.7.3 Equipment/apparatuses

Batch reactor vessels, see (Section 2.6.1.2 and Figure 2-6) was used to carry out the hydrothermal decarboxylation process. Mettler Toledo (AB204 – S, Mettler-Toledo International Inc.) was used for measuring the weights of reactants, catalysts and products of hydrothermal decarboxylation reaction. Gallenkamp vacuum oven was used for drying the materials recovered post hydrothermal decarboxylation reaction. Hewlett Packard 5890 Series II Oven (5890 Series II plus Gas Chromatograph, equipped with input bench digital thermometer and 12way Type K thermocouple selector switch and Type K thermocouple) was used for carrying out the decarboxylation reaction at the desired temperature. Water bath (Julabo SW22 Shaking, temperature control +20°C to +99°C) was used to raise the temperature of the materials post hydrothermal decarboxylation in order to ensure complete separation.

Barnstead Lab-Line 2052-1 Mult-Blok Heater (Thermo Scientific) was used for drying of the decarboxylation products.

2.7.4 Experimental procedures for hydrothermal decarboxylation of palmitic acid, oleic acid and lipid extract

2.7.4.1 Determining the effect of temperature and catalyst type on conversion, yield, and selectivity of the hydrocarbon products

This was performed at varied temperatures of 290, 300 and 330°C using 100 mg of palmitic acid or oleic acid samples, 15 mL of distilled water and 20 mg of Pd/C in a batch reactor for 4h, according to the method, described in Section 2.6.1.3. The process was repeated under the same experimental conditions using Pt/C. The experiments were conducted in triplicate. At the end of the reaction, the products were collected in pre-weighed 50 mL centrifuge tubes and dried in the oven at 50°C to a constant weight. After which, the hydrocarbon products were separated from the catalysts in vacuum filtration apparatus containing filter paper with pore size of 11 µm, using 10 mL of hexane, having heated the products in a water bath at 80°C for 2 min. The recovered organic phase containing the hydrocarbons was dried to a constant weight at 50 °C in order to ensure that all the hexane was removed. The hydrocarbons were then analysed using high temperature gas chromatography coupled to flame ionisation detector (HT-FID) according to the method described in Section 2.9.3.3.

2.7.4.2 Determining the effect of residence time on conversion, yield, and selectivity of the hydrocarbon products

After analysing the hydrocarbon products obtained from Section 2.7.4.1, it was discovered that higher molar yield of pentadecane was obtained at 290°C using palmitic acid under the reaction conditions considered. Therefore, the effect of residence time was explored at this temperature 290°C, 100 mg of palmitic acid and oleic acid, 20 mg of Pd/C catalyst and 15 mL of water. The

process was repeated using Pt/C catalyst and the experiments were carried out in triplicate. The hydrocarbon products were recovered as presented in Section 2.7.4.1.

2.8 TITRIMETRIC METHOD

This method was used to determine the acid value and percentage free fatty acid (%FFA) of the lipid extract and Sunflower vegetable oil. These were evaluated, according to AOAC (2005) Modified, which was developed from AOAC Official Method 940.28 Fatty Acids (Free) in Crude oil (AOAC, 2005). This method was obtained by scaling down the materials: 7.05g of oil, 50 mL of ethanol, 0.1 and 0.25M NaOH stated in the AOAC (2005) to 40 mg, 25 mL of ethanol, 0.57 and 1.42 mM NaOH, respectively, because of the limited amount of the lipid extract available. The acid value of the Sunflower oil was determined in order to evaluate the efficacy of the method developed. The solutions of NaOH were standardized using potassium hydrogen phthalate (KHP), to ascertain its actual concentration, as it is known to be deliquescent and thus, unstable on exposure to the environment.

(1). Samples

Lipid extract was obtained from activated sludge (Finham STW, batch 2) using the Folch method and refined Solesta sunflower oil (100%) (purchased from ALDI Supermarket, Birmingham, UK) was used as a model oil with known percent of acid value to evaluate the efficacy of the method developed.

(2). Materials

Phenolphthalein was used as an indicator, NaOH pellet ($\geq 98\%$) as a base, KHP for standardizing the concentration of NaOH and they were purchased from Sigma-Aldrich.

(3). Equipment/apparatuses

Mettler-Toledo, analytical balance (AG204, Mettler-Toledo International Inc.) was used for measuring the amount of the vegetable oil and lipid extract. Burette was used for delivering NaOH and retort stand was for holding the burette.

2.8.1 Experimental procedures

2.8.1.1 Procedure for determining the acid value of sunflower oil using AOAC Official Method 940.28 Fatty Acids (free) in Crude Oils

A well-mixed 7050 mg of sunflower oil was put into a 250 mL Erlenmeyer flask and a previously neutralized 50 mL ethanol with 2 drops of phenolphthalein and 20 mL of 0.1 M NaOH were added to the flask. The resulting solution was titrated with 0.25 M NaOH while swirling the Erlenmeyer flask vigorously until a permanent faint pink colour appeared. The acid value was calculated using Equation 2-6 as a percentage free fatty acids, % (FFA) expressed as oleic acid. The titration was done in triplicate and the average titre value of 0.25 M NaOH determined and was used in the calculation of amount of the oleic acid that was present in the sunflower oil.

$$\% \text{ FFA (as oleic)} = (\text{Mass of oleic acid} / \text{Average Mass of sunflower oil}) \times 100$$

$$\text{Acid value (AV)} = \% \text{ FFA (as oleic)} \times 1.99 \quad \text{Equation 2-6}$$

(Note: the mass of oleic acid was calculated using the titre values of NaOH obtained by titration)

2.8.1.2 Procedure for determining the acid value of Sunflower oil and lipid extract using AOAC (2005) Modified

The procedure presented in Section 2.8.1.1 was adapted, although, 40 mg of Sunflower oil and 25 mL of ethanol were used. Also, 0.57 mM and 1.42 mM NaOH were used in place of 0.1 M and 0.25 M NaOH, respectively. The experiment was repeated using 40 mg of lipid extract from activated sludge.

2.9 INSTRUMENTATION

This covers the equipment and methods used in analysing the products of decarboxylation of free fatty acids and lipid, and lipid composition in activated sludge, including the fatty acid determination through ester production. The equipment used were thin layer chromatography (TLC), high performance thin layer chromatography (HPTLC) and gas chromatography (GC). TLC, HPTLC and GC are presented in the Appendix, Section 2.9.1 and Section 2.9.2, respectively. The TLC was presented in the Appendix as an additional method used to enhance understanding of HPTLC.

2.9.1 High performance thin layer chromatography (HPTLC)

High performance thin layer chromatography (HPTLC) operates on the same principles as the conventional thin layer chromatography (TLC), except that it has many features which improves the quality of the results obtained from the chromatographic processes, and the speed of analysis (Arup et al., 1993). Unfortunately, the features that make HPTLC more attractive, also increases the cost of the operation, as more sophisticated instruments are required. These include sample application instrument, automatic developing chamber, TLC Scanner, TLC visualizer, chromatographic immersion device, TLC plate heater and software. Although, all the instruments are very important, especially, if high quality and reproducibility are required, HPTLC is flexible and could be combined with some of the operations of the conventional TLC, but, quality might be compromised. For instance, instead of using a more sophisticated immersion device and TLC plate heater, the same operations applied in the conventional TLC, can be used, when this happens, the capital cost will be reduced. The HPTLC automatic sample application instrument uses a technique called spray-on technique. This technique allows the application of the sample on the chromatographic plate in bands, the advantage of which is that more volume of the sample would be applied compared to conventional TLC which supports

spot wise loading of samples (CAMAG, 2013). The band wise application of the sample also helps to ensure that the highest resolution is achieved and that photodensitometry evaluation is carried out by aliquot scanning, thereby, giving rise to a more accurate quantitative result. The use of an automatic developing chamber is also one of the features as stated before which makes HPTLC to be more attractive than the conventional TLC. This chamber helps to ensure that reproducible chromatograms are obtained through the use of the option of humidity control. It requires less volume of the solvent system and hence, reduction in the overall cost of operation and more safety is ensured compared to using the conventional TLC (CAMAG, 2013). The use of this chamber, also, ensures that small dimensions of the TLC/HPTLC plates such as 20 x 10cm and 10 x 10 cm are used, the advantages of which are less consumption of solvent and less separation time (Arup et al., 1993, Sherma, 2003).

2.9.1.1 Analyses of lipid extract using HPTLC

(1). Sample

This was the lipid extract obtained from activated sludge biomass (Finham STW, batch 2) using Folch method

(2). Materials

The standards used to analyse the neutral lipids were cholesterol (99 + % GC), cetyl palmitate, 1-monooleoyl-RAC-glycerol, diolein, glyceryl trioleate and oleic, and they were all purchased from Sigma-Aldrich. While those used to analyse the phospholipids were L - α - phosphatidylcholine from egg yolk, type XV1-E (\geq 99%, TLC, lyophilized powder), 1, 2 - diacyl-sn-glycero-3-phospho-L-serine (\geq 97% TLC), L- α -phosphatidylinositol from glycine max (soybean) and phospholipid mixture (for HPLC from soybean), and they were purchased from Sigma-Aldrich. Petroleum ether 60 – 80°C (Fisons Scientific Equipment), diethyl ether (Fluka Analytical; Sigma – Aldrich), acetic acid (purum, Sigma-Aldrich), chloroform (reagent

plus, 99.8), methanol (99% +, Fisher Scientific) and ammonia solution (35%) (Analytical reagent grade, Fisher Scientific) were used solvents for lipid analyses.

(3). Equipment/apparatuses

The Gallenkamp vacuum oven was used for charring the chromatograms on the HPTLC plate for visualising the lipids. CAMAG Linomat 5 was used for bandwise sample application. CAMAG Automatic Development Chamber ADC2 was utilised for separating the lipid components using a suitable solvent system. CAMAG TLC Visualizer was used to obtain the visual image of the samples and standards. CAMAG TLC Scanner 4 was used for densitometric assessment of the chromatograms. The winCATS software was used to operate all the CAMAG TLC/HPTLC instruments. Sartorius Research Electronic Semi-Microbalance (Analytical products Ltd, England) was used for measuring the weight of lipids. Hamilton (701SNR 10 μ L, Sigma-Aldrich) was used for measuring appropriate volume of lipid concentration for loading on the HPTLC plate.

2.9.1.1.1 Experimental procedure for the qualitative analysis of lipids

The method presented in Table 2-5 was developed using winCATS software and was employed for the lipid analysis. The amount of lipid extract and standards loaded on the same plate were 25 μ g and 2 μ g, respectively, which were prepared using chloroform/methanol (2/1, v/v). Neutral lipids were eluted using 100 mL petroleum ether/diethyl ether/acetic acid (80/20/1, v/v/v) (Mangold, 1969) as the solvent systems. The plate containing the separated lipids was dried in the CAMAG Automatic Development Chamber ADC2. Lipids were derivatized by dipping the plate in a pool of 100 mL of 5% sulphuric acid in ethanol and visualized after charring in the oven at 180°C for 5min (Appendix 8.2.1.1.1 (3a)). The plate was further scanned in order to obtain photodensitometric data which would aid the identification of the

lipids and their subsequent quantification. Data obtained were evaluated to get the peak characteristics of the lipid bands.

The above procedure was followed to analyse the phospholipids using standards L - α phosphatidycholine, L - α – phosphatidylethanolamine, L - α - phosphatidylinositol and 1, 2 – Diacyl-sn-glycero-3-phospho-L-serine and chloroform/methanol/28% ammonia (65/25/5, v/v/v) (Rouser et al., 1969) was used as the solvent system. The experiment was done in triplicate and retention factors (Rf) of the lipids are presented in Table 2-6.

Table 2-5: example of method developed using WinCATS software for the CAMAG TLC/HPTLC neutral lipid analysis

Parameter	Description
Stationary phase	Glass HPTLC plate silica gel 60 F254, 20 x 10 cm, HX304850, Merck KGaA, 64271 Darmstadt
	Drying device (oven): 120°C at 20 min
Definitions-Quantitative	Calibration mode: multi-level, evaluation via peak height , statistics : CV
Sample application-Linomat 5	Syringe size:100 µL, band length: 6.0mm, application position Y: 10mm, first application position X:15mm and distance between tracks: 14.1 mm
Development-ADC 2	Pre-drying of the plate was enabled. Tank saturation was also enabled. Tank saturation time:5min, tank filling volume:25 mL, solvent: petroleum/diethyl ether/acetic acid (80/20/1, v/v/v) Development: Plate preconditioning time:5min, filling volume:10 mL, solvent: petroleum/diethyl ether/acetic acid (80/20/1, v/v/v), migration distance:85 mm, drying time:5min
Detection-Scanner 3	Scan mode: single wavelength, scan display scaling: automatic, track optimization (passes:7 and spacing: 0.5mm), spectrum mode: none, link parameters to previous TLC steps: checked Rf positions (gray if linked to previous TLC steps): application position:10mm and solvent front position:85mm
	Scan settings Slit dimension: 4 x 0.30 mm, Micro Optimize Optical system for maximum: light, scanning speed: 20mm/s, Data resolution: 100µm/step Measurement: wavelength (600 nm), lamp (D2 & W), measurement type (remission), measurement mode (absorption), optical filter (second order), detector mode (automatic), Y-position for 0 adjust (5mm), Track# for 0 adjust (1), Track start for quick scan (automatic), Track end for quick scan (automatic), Track # for quick scan (automatic), analog offset (10%) and sensitivity: automatic
	Filter factor: Savitsky-Golay 13; baseline correction: lowest slope Peak thresholds Minimum slope: 7; minimum height: 24AU; minimum area: 522 AU; Maximum height: 436AU Integration limits Track start position: 5mm; track end position: 75mm Display scaling: automatic
Evaluation-Quantitative	Evaluated all the tracks
Documentation-Visualizer	Plate states to be documented: Developed plate (254 nm, 366 nm, white R, white RT, white T) Derivatized plate (254 nm, 366 nm, white R, white RT, white T) Default settings Frame: -2mm, resolution: high, save: lossy (JPG), exposure: auto, exposure time: 30 ms, gain: 1:00 Digital Camera: DXA252, 307051208

Table 2-6: Retention factors of lipids obtained from the qualitative analysis of the lipids using CAMAG TLC/HPTLC (solvent system for neutral lipid was petroleum ether/diethyl ether/acetic acid (80/20/1, v/v/v) and that for phospholipid was chloroform/methanol/28% ammonia (65/25/5, v/v/v))

S/N	Lipid standards		Retention factor	
			Standards	Lipids extract
1	Neutral lipids	1- oleoyl glycerol	-0.02	-0.02
		1,2-diacyl glycerol	0.06	0.06
		1,3-diacyl glycerol	0.08	0.08
		Cholesterol	0.11	0.11
		Oleic acid	0.18	0.18
		Cety palmitate	0.51	0.51
2	Phospholipids	L-alpha-phosphatidylcholine (PC)	0.27	0.27
		L-alpha-phosphatidylethanolamine (PE)	0.34	0.34
		L-alpha-phosphatidylinositol (PI)	0.07	0.07

2.9.1.1.2 Experimental procedure for the quantitative analysis of lipids

The quantitative analysis of lipids was carried out according to the method described in Section 2.9.1.1.1. The amount of lipid extract loaded on the HPTLC plate was 25 µg and those of the standards for developing calibration curves are presented in Table 2-7. The calibration curves were developed by plotting the mass load of the lipid standards against the corresponding area of peaks obtained from the analysis, see Table 2-8. The lipids identified were quantified using the correlations obtained from the calibration curves, see Table 2-8 and Figure 2.8.

Table 2-7: mass load of the lipid standards used for developing calibration curves for quantifying the lipid extract

S/N	Lipid standards	Lipid mass load (µg)					
1	1,2-Diacyl glycerol	0.6	0.7	0.8	4	8	
2	1,3-Diacyl glycerol	0.5	0.6	0.7	0.8	1	6
3	Oleic acid	0.5	0.6	0.7	0.8	1	6
4	Cetyl palmitate	0.6	0.7	0.8	1	6	10
5	Cholesterol	0.6	0.7	0.8	1	6	
6	Phospholipid (PC)	0.7	0.8	1	2	3	4

Table 2-8: parameters for quantifying the lipids extract in the activated sludge

Peak area (Au)						
Standards						
Lipid load (ug)	Phospholipids	1,2-diacyl glycerol	1,3 -diacyl glycerol	Oleic acid	Cetyl palmitate	Cholesterol
0.5			1411.6	1594.8		
0.6		567.9	1250.4	1299.0	4144.9	2407.5
0.7	1812.9	675.0	1622.8	1891.2	4677.6	2913.3
0.8	1854.0	787.1	1575.5	2038.2	4907.5	2980.3
1	2357.7		1897.5	2348.2	5891.4	3303.0
2	3903.7					
3	6929.0					
4	9618.3	2274.1				
6			6106.5	7544.6	19699.7	8418.8
8		4090.5				
10				10683.3	31009.7	
Model: y =	0.0007x - 0.5436	0.0021x - 0.7522	0.0012x - 1.0642	0.001x - 1.1613	0.0004x - 0.9549	0.0009x - 1.9378
R ²	0.9984	0.9989	0.9964	0.9928	0.9997	0.9946

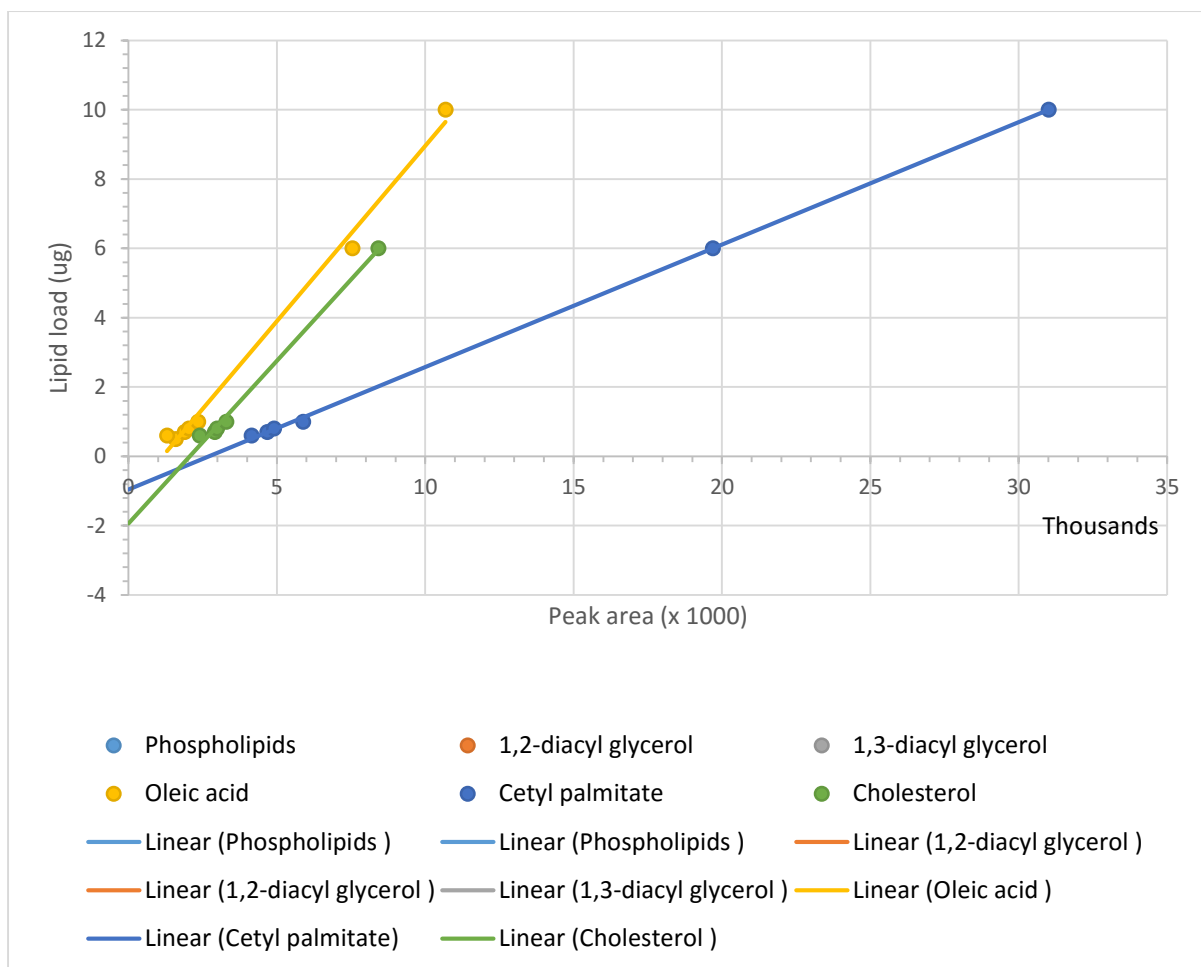


Figure 2-8: Standards calibration curves for quantifying the lipids extract from the activated sludge

2.9.1.2 Monitoring the methanolysis reaction of the lipid extract leading to FAMES (biodiesel) production by HPTLC

The methanolysis reaction was monitored using the degradation of acylglycerol, obtained from Section 2.4.4. The products of this reaction were analysed for acylglycerol (1, 2-diacylglycerol, 1, 3-diacylglycerol and monoacylglycerol) using HPTLC, according to the experimental procedure presented in Section 2.9.1.1.1. The amount loaded on the HPTLC plate was 8 μ g from the products produced at residence times 0.08, 0.25, 0.5, 1, 12, 24 and 72 h, respectively. The solvent system used was 100 mL petroleum ether/diethyl ether/acetic (80/20/1, v/v/v) (Mangold, 1969). The amount of the acylglycerols were calculated using the models presented

in Table 2-8 and plotted against the residence time. The analysis was conducted in triplicate and average results reported.

2.9.2 Gas chromatography (GC)

2.9.2.1 Analyses of fatty acid methyl esters (FAMES)

2.9.2.1.1 Qualitative analysis of the FAMES produced from the lipid extract from activated sludge

The FAMES produced from methanolysis of lipid extract using acidic catalysts were analysed by gas chromatography (GC). The GC equipment used was Agilent Technologies 6850 Network GC System coupled with flame ionization detector (FID). The GC column used was Agilent DB-5 capillary column of 30m x 0.25mm x 0.10µm. The carrier gas was helium gas, hydrogen gas and air were used to ignite and sustain the detector flame while nitrogen gas was used as a makeup gas. The GC method used for the FAMES analysis was that presented by Ong et al. (2013), although with modification. Thus, unlike Ong et al. (2013) that used a constant helium linear velocity of 40 cm/s, injector temperature of 250°C, maximum oven temperature of 250°C held at 8min, the current GC method utilized 1.2 mL/min, 290°C and 280°C held for 8min for the helium linear velocity, maximum injection and oven temperatures, respectively. Also, Ong et al. (2013) used Equation 2-6 to calculate the yield percentage of the FAME (wt%) while the current method used the equation that was adapted from Kaiser and Debbrecht (1977), shown in Equation 2-7.

$$FAME \text{ (wt\%)} = 100 \left(\frac{\sum A_{ME} - A_{int}}{A_{int}} \times \frac{V_{int} C_{int}}{m} \right) \quad \text{Equation 2-7}$$

Where: A_{ME} = area of the peak of the methyl ester, A_{int} = area of the internal standard, C_{int} = concentration of the internal standard, V_{int} = volume of the internal standard, m = weight of the sample

Furthermore, in the current analysis, the volume of the sample and FAME standards that were injected into the capillary column was 1 μ L through the injector which was heated to a temperature of 280°C and which was on the splitless mode. The carrier gas: helium gas moved the sample at a flow rate of 1.2 mL/min through the capillary column which was installed inside the GC oven, heated initially at 50°C and held for 2min, then ramped at 10°C/min to a temperature of 250°C, which was held for 8min, before ramped again at 20°C/min to a temperature of 290°C and finally held for 8min. The sample and FAME standards eluted were detected using the flame ionization detector fixed at a constant temperature of 300°C. The FAMES obtained from the sample were identified by matching their respective residence times with those of the FAME standards which were also prepared using the same method of derivatization as was used with the lipid extract. The FAME standards used included capric acid methyl ester, myristoleic acid methyl ester, palmitic acid methyl ester, palmitoleic acid methyl ester, stearic acid methyl ester, oleic acid methyl ester, linoleic acid methyl ester, linolenic acid methyl ester, arachidic acid methyl, erucic acid methyl ester, heptadecanoic acid methyl ester, cis-8, 11, 14-eicosatrienoic acid methyl ester, nervonic acid methyl ester, cis-9-tetradecenoic acid methyl ester and petroselinic acid methyl ester. 400 ng/ μ L of each fatty acid identified was mixed together and 1 μ L injected into the GC, in order to obtained the FAMES profile. This was validated by running 1 μ L of the same concentration using a Grain Fatty Acid Methyl Ester Mix on the GC (Figure 2-9).

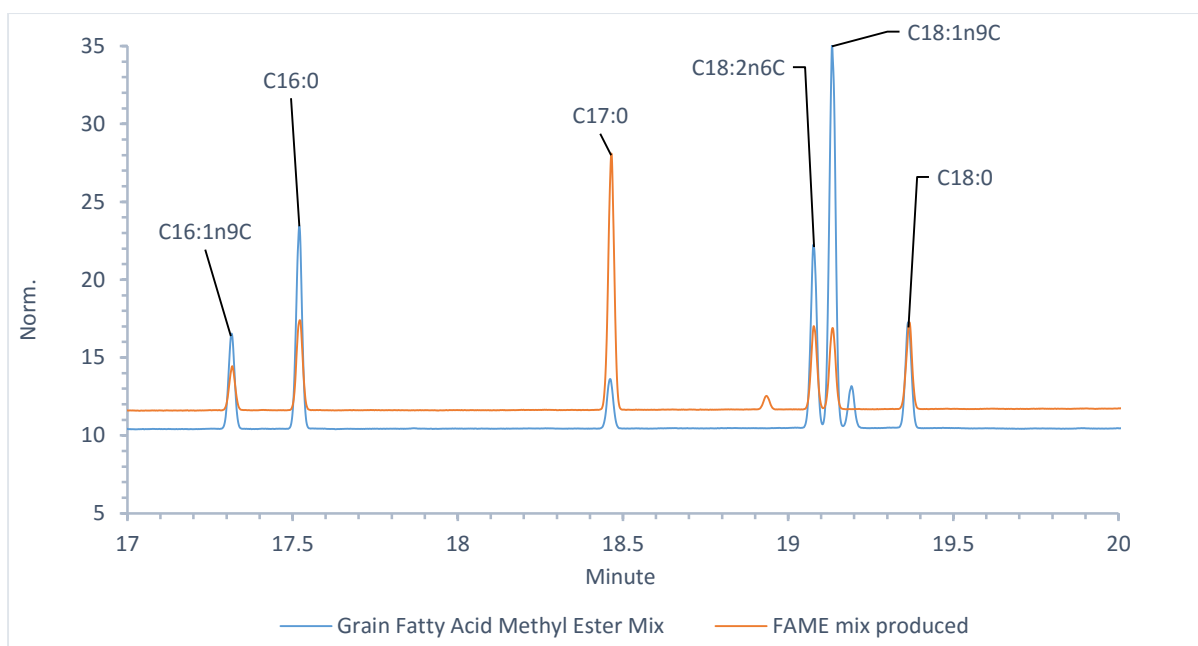


Figure 2-9: typical profile of the FAME standards obtained from the methanolysis of fatty acid standards and grain fatty acid methyl ester mix (purchased from SUPELCO) using gas chromatography (Agilent Technologies 6850 Network GC system) (C16:1n9C = palmitoleic acid methyl ester, C16:0 = palmitic acid methyl ester, C17:0 = heptadecanoic acid methyl ester (internal standard), C18:2n6C = linoleic acid methyl ester, C18:1n9C = oleic acid methyl ester and C18:0 = stearic acid methyl ester)

2.9.2.1.2 Quantitative analysis of the FAMES produced from lipid extract

The fatty acids identified in the activated sludge were quantified using GC, according to the method described in Section 2.9.3.1. The amounts of FAME standards injected into the GC are presented in Table 2-10. These were plotted against the normalised area of the peaks obtained from the GC to get the models and coefficient of correlations (Table 2-10 and Figure 2-10). With these models, the fatty acids in the lipid extract were quantified. The analysis was conducted in duplicate and the average values of the residence time and peak area were used. The percentage yield of the FAMES were determined using Equation 2-8 (Kaiser and Debbrecht, 1977).

$$FAMES \% = \sum \frac{\text{Area of FAMES peak}}{\text{Area of internal standard peak}} \times \frac{\text{Weight of the internal standard}}{\text{Weight of the sample}} \times 100$$

Table 2-9: calibration data for quantifying the FAMES produced from the lipid extract

FAME load (µg)	Palmitoleic acid methyl ester	Palmitic acid methyl ester	Linoleic acid methyl ester	Oleic acid methyl ester	Stearic acid methyl ester
Peak area ratio = area of the FAMES/area of the internal standard					
0.10	0.38	0.59	0.49	0.5	0.45
0.20	0.71	1.04	0.98	1.03	
0.30	1.09		1.51	1.51	
0.40	1.61		1.98		1.77
0.50	2.01	2.39			2.23
0.60				3.03	
0.70			3.89	3.58	3.09
0.80					3.47
0.90		4.04	5.24		
1.00		4.48	5.77	5.06	
Model:					
y =	0.239+0.0228x	0.2323x-0.0425	0.1665x+0.0419	0.1972x-0.0001	0.2303x-0.0071
R ²	0.9941	0.9997	0.9977	0.9999	0.9995

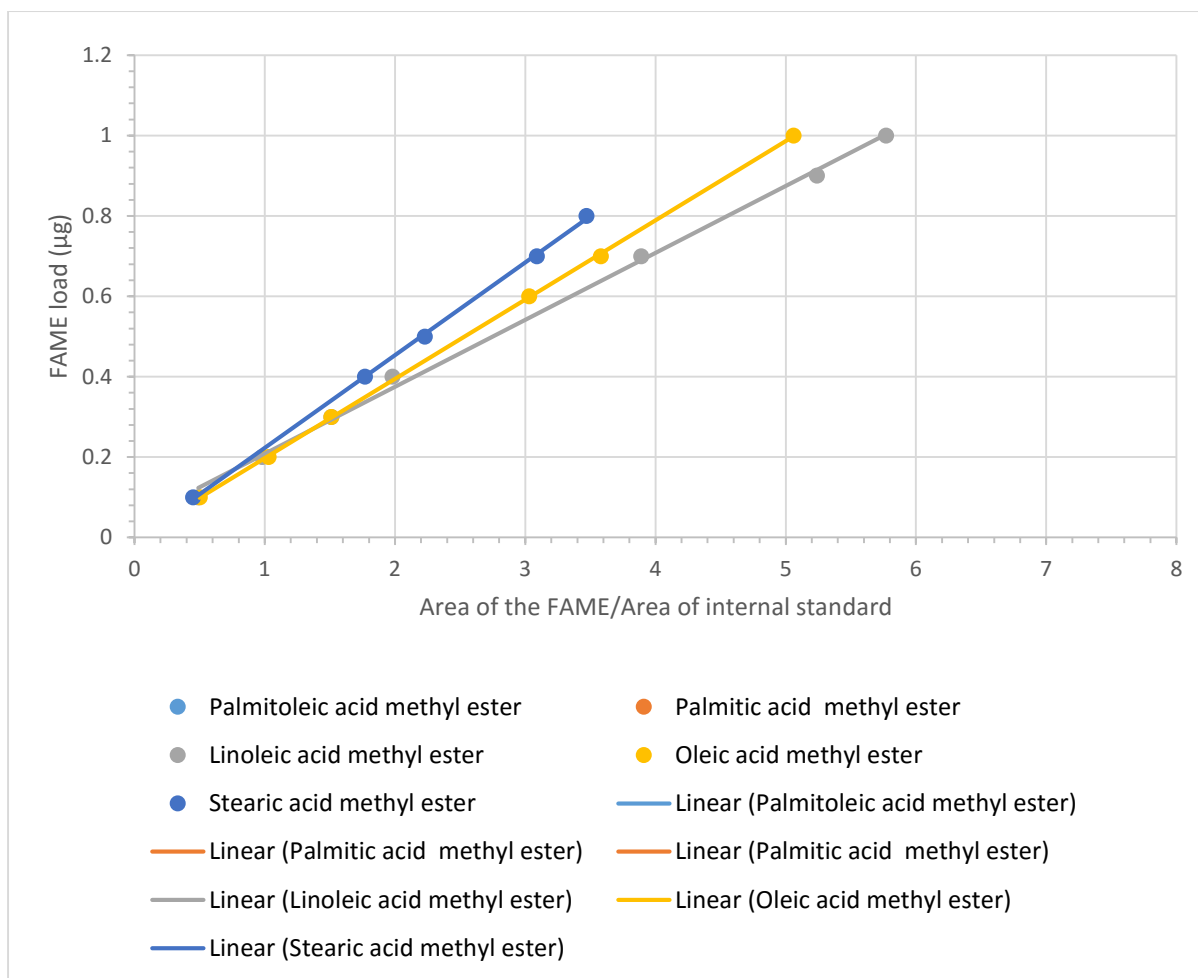


Figure 2-10: calibration curves of the FAME standards obtained from the GC-FID analysis

2.9.2.2 Analyses of lipid extract from subcritical water mediated extraction

2.9.2.2.1 Samples

These were the lipids extracted from the liquid and solid activated sludge biomass post subcritical water mediated extraction (DoE) at different experimental conditions (runs), see Section 2.6.3.

2.9.2.2.2 Materials and equipment

The standards used to analyse the neutral lipids were cholesterol, cetyl palmitate, 1-monooleoyl-RAC-glycerol, diolein, linoleic, stearic acid, behenic acid, decanedioc acid, glyceryl trioleate, heptadecanoic acid (internal standard) and oleic acid, and they were all

purchased from Sigma-Aldrich. While those used to analyse the phospholipids were L – α – phosphatidylcholine from egg yolk, type XV1-E, 1, 2 – diacyl-sn-glycero-3-phospho-L-serine, L- α -phosphatidylinositol from glycine max (soybean) and they were purchased from Sigma-Aldrich. Hexane was used as the solvent for dissolving the lipids.

Agilent Technologies 6850 Network GC System equipped with (Agilent 6850 series auto sampler, FID detector and DB-5HT column (J & W 123-5711, 15m x 320 μ m x 0.1 μ m; 400°C max temperature) was used to analyse the lipid samples. Sartorius Research Electronic Semi-Microbalance (Analytical products Ltd, England) was used to measure the weight of the lipids

2.9.2.2.3 Experimental procedures

(i) Qualitative analysis

Concentrations of 5 μ g/ μ L of lipids (lipid extract and standards) were prepared using hexane, and heptadecanoic acid as the internal standard. 1 μ L of each lipid was injected into the GC column in duplicate and analysed. The injection and detector temperatures were 325°C, H₂ air flow rates and makeup flow of 30 mL/min, 300 mL/min, and 20 mL/min respectively. The GC was programed to start at 40°C and held for 5min, ramped to 290°C at 15°C/min with a total run time of 45 min. The lipid compositions were identified by matching their respective residence times with those obtained from the standards.

(ii). Quantitative analysis

The calibration curve of each lipid standard was obtained by plotting the amount injected into the column: 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, 2.5 and 5.3 μ g against the peak area ratio. The correlations obtained were used to quantify the lipid composition in the activated sludge. A sample of the calibration curves is presented in Figure 2-11.

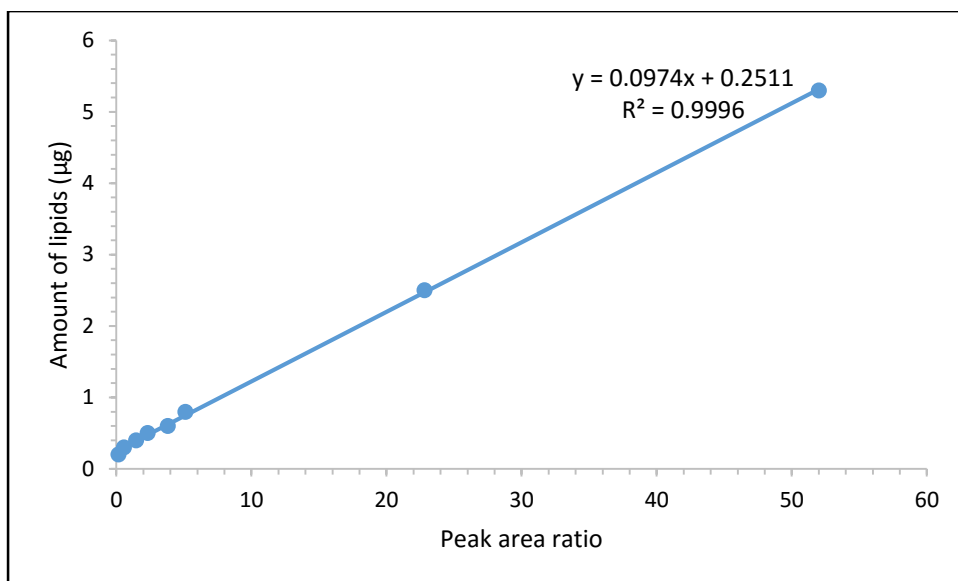


Figure 2-11: an illustration of a calibration curve using palmitic acid standard (peak area ratio = peak area of lipid / peak area of the internal standard). The peak areas were obtained from the GC analysis of lipids

2.9.2.3 Analyses of the products of hydrothermal decarboxylation of palmitic acid, oleic acid, and lipid extract from activated sludge

2.9.2.3.1 Samples

The hydrocarbon products: pentadecane and heptadecane were obtained from the hydrothermal decarboxylation of palmitic acid and oleic acid, respectively. Palmitic acid, oleic acid and lipid extract (from activated sludge, Minworth batch 2) were used as the feedstocks.

2.9.2.3.2 Materials and equipment

N-pentadecane and n-heptadecane (99%) were purchased from Alfa Aesar and MP Biomedicals and were used as hydrocarbon standards for the analyses of the products. Eicosane (purum; 97%, GC) was bought from Fluka Chemika and was used as the internal standard. Hexane and acetone were used as the solvents for preparing pentadecane and heptadecane for analyses.

Agilent Technologies 6850 Network GC System equipped with (Agilent 6850 series auto sampler, FID detector, DB-5HT column (J & W 123-5711, 15m x 320 μ m x 0.1 μ m; 400°C max temperature) was used to analyse the hydrocarbon samples. Sartorius Research Electronic Semi-Microbalance (Analytical products Ltd, England) was used to measure the weight of the lipid extract, palmitic acid, oleic acid and the recovered hydrocarbon products for analyses.

2.9.2.3.3 Experimental method

(ii) Qualitative analysis

A concentration of 2 μ g/ μ L of pentadecane and products recovered post hydrothermal decarboxylation of lipid extract were prepared using hexane, and that of heptadecane using acetone, and eicosane was employed as the internal standard. 1 μ L of each substance including the feedstocks was injected into the GC column in duplicate and analysed, using the same GC method described in Section 2.9.3.3.3. The hydrocarbon obtained from the hydrothermal decarboxylation of lipid extract and feedstocks were identified by matching their respective residence times with those of the standards.

(ii). Quantitative analysis

The calibration curve of each hydrocarbon standard was obtained by plotting the amount injected into the column: 0.05, 0.1, 0.3, 0.5, 0.8 and 1 μ g against the peak area ratio. The correlations obtained were used to quantify the hydrocarbon products from the hydrothermal decarboxylation of the feedstocks. A sample of the calibration curves is presented in Figure 2-12. The feedstocks were quantified using the method described in Section 2.9.3.3.3 (ii).

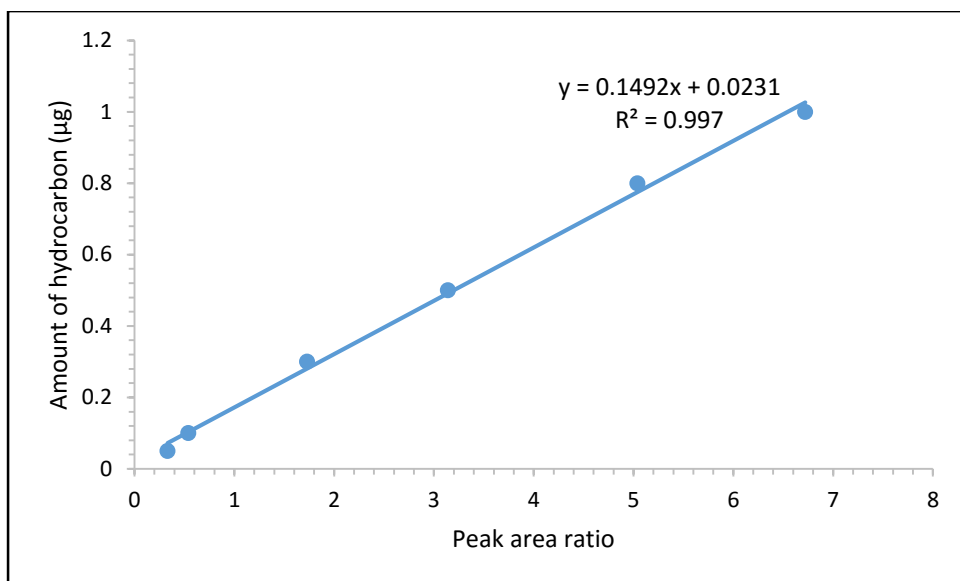


Figure 2-12: an illustration of a calibration curve using heptadecane standard (peak area ratio = peak area of the substance/peak area of the internal standard)

(iii). Calculations

The molar yield and selectivity of the hydrocarbon products, and conversion of the feedstocks were determined using the following equations

$$(a). \text{Product molar yields (\%)} = \frac{N_p}{N_o} \times 100 \quad \text{Equation 2-9}$$

$$(b). \text{Conversion(\%)} = \frac{N_o - N_p}{N_o} \times 100 \quad \text{Equation 2-10}$$

$$(c). \text{Selectivity(\%)} = \frac{\text{Product yield}}{\text{conversion}} \times 100 \quad \text{Equation 2-11}$$

Where N_p = number of moles of product recovered; N_o = initial number of moles of reactant

3 CHAPTER 3 CHARACTERIZATION OF

BIOMASS AND LIPID CONTENT IN ACTIVATED

SLUDGE

3.1 INTRODUCTION

Bioenergy such as biodiesel, renewable diesel, bioethanol and biobutanol have been gaining popularity as potential sources of renewable energy, capable of displacing or substituting the conventional fossil fuels (Agarwal, 2007, Knothe, 2008, Lapuerta et al., 2007). For biodiesel production, feedstocks are sourced from pure vegetable and seed oils (Hass and Foglia, 2005, Mondala et al., 2009). Different countries use various feedstocks for biodiesel production. For instance, US utilises soya bean oil, Europe rapeseed and sunflowers oils, Malaysia and Indonesia palm oil and Philippines coconut (Korbitz, 1999). These feedstocks contribute to 70 – 85% of the total biodiesel production cost, as a result make it non-competitive to diesel fuel (Hass and Foglia, 2005, Kargbo, 2010). The use of vegetable oils in biodiesel production might probably increase its price, and lead to scarcity if demand and supply are not met, due to interference with the quantity required for human consumption. In order to solve this problem of cost, some researchers have investigated the potential use of alternative cheap feedstocks such as used frying oil, microalgae, oleaginous microorganisms, animal fats and non-edible oil (jatropha, castor, neem, karanja) (Revellame et al., 2010, Revellame et al., 2012b). Unfortunately, used vegetable oil and animal fats contain high free fatty acid and are often adulterated, these pose a production problem and might result to a biodiesel with inconsistent quality (Vyas et al., 2010). The use of non-edible oils needs cultivation which in turns is energy

demanding and requires large expanse of land which might compete with other uses (Revellame et al., 2010). At the moment, activated sludge is being considered as a potential feedstock that has the capability to reduce the cost of biodiesel production to the level where it can have a comparative advantage over petroleum diesel (Revellame et al., 2010). This is because, it has a high lipid content and is readily available (Huynh et al., 2012). The constituents of activated sludge, include microorganisms, colloidal matter, organic polymers, suspended solids, mineral particles and cations which are generated from the biological treatment of wastewater (Metcalf and Eddy, 2003, Mondala et al., 2009). Numerous amounts of it are produced annually and are combined with other sludges from primary and tertiary wastewater treatment plants. The combination of these is called sewage sludge and is available free of charge or disposed with incentive (Dufreche et al., 2007, Fytili and Zabaniotou, 2008). Before sewage sludge is disposed, it has to be treated and thickened in order to reduce the cost of transportation (White and Plaskett, 1981). Sludges are disposed in oceans, landfills, used for agricultural purposes or for generating energy through incineration. Due to some legislations on sludge disposal, there is a need to develop a strategy for sustainable treatment. These involve integrated use of sludge as biomass for energy production, recovery and reusing of the valuable components, and minimising the adverse impact of sludge on human, animals and environment (Rulkens, 2008, Fytili and Zabaniotou, 2008, Siddiquee and Rohani, 2011). The methods for recovering values from sludge include anaerobic digestion, incineration, gasification and pyrolysis, supercritical wet oxidation, solvent and subcritical fluid extraction and hydrothermal treatment, giving rise to products such as methane, lipid, inorganic compounds and protein (Kalogo and Monteith, 2008).

In this work, activated sludge was investigated as a potential feedstock for oleochemical and bioenergy (biodiesel and renewable diesel) production. This means of creating values also lends itself as a sustainable sludge disposal strategy (Mondala et al., 2009, Pastore et al., 2013).

It is expected that the results obtained from this Chapter would form a bedrock for further investigation on increasing lipid yields and optimisation, as low percentages have been reported by various researchers.

Activated sludge slurry was obtained from two sewage treatment works (STW) in the UK, at different seasons. This was characterized to determine its total solid content and how the biomass is partitioned as total suspended solid (TSS) and total dissolved solid (TDS), using filtration and drying methods. The efficacy of using Soxhlet and Folch methods to maximise lipid yield from the solid biomass was investigated. The lipid derived was characterised using thin layer chromatography (TLC) and high performance thin layer chromatography (HPTLC). The fatty acid content was determined by gas chromatography (GC) from the fatty acid methyl esters (FAMES) produced through the methanolysis of lipid extract using hydrochloric acid (HCl) and boron trichloride (BCl_3). The yields are either presented in terms of lipid extract or dry activated sludge (wt/wt) %. Otherwise stated, the measurements are done in triplicate ($n = 3$) with the average value and standard error of the mean (SEM), presented.

3.2 RESULTS AND DISCUSSIONS

3.2.1 *Characterization of the activated sludge*

This was done in order to determine the amount of biomass in the activated sludge slurry which was available for extraction of lipid, by filtration and drying, see Section 2.2. For us to obtain a total lipid from activated sludge, we considered it necessary to investigate if there was any biomass in the filtrate (water), as many researchers have reported discarding it.

3.2.1.1 Biomass partitioning within the activated sludge

The biomass in the activated sludge (AS) was partitioned into TSS and TDS in order to assess the lipid content in both parts. This was done by filtering the activated sludge slurry and drying the residue and filtrate to obtain TSS and TDS, respectively, as described in Section 2.2.1.1.1. The results were measured gravimetrically and presented in terms of TSS, TDS and TS (total solid), see Figure 3-1 for an illustration of biomass partitioning using activated sludge obtained from Finham STW (batch 1). Figure 3-1 shows that TSS was greater than TDS and that the total biomass content in activated sludge was 0.38 % of 5130 g activated sludge supplied. This

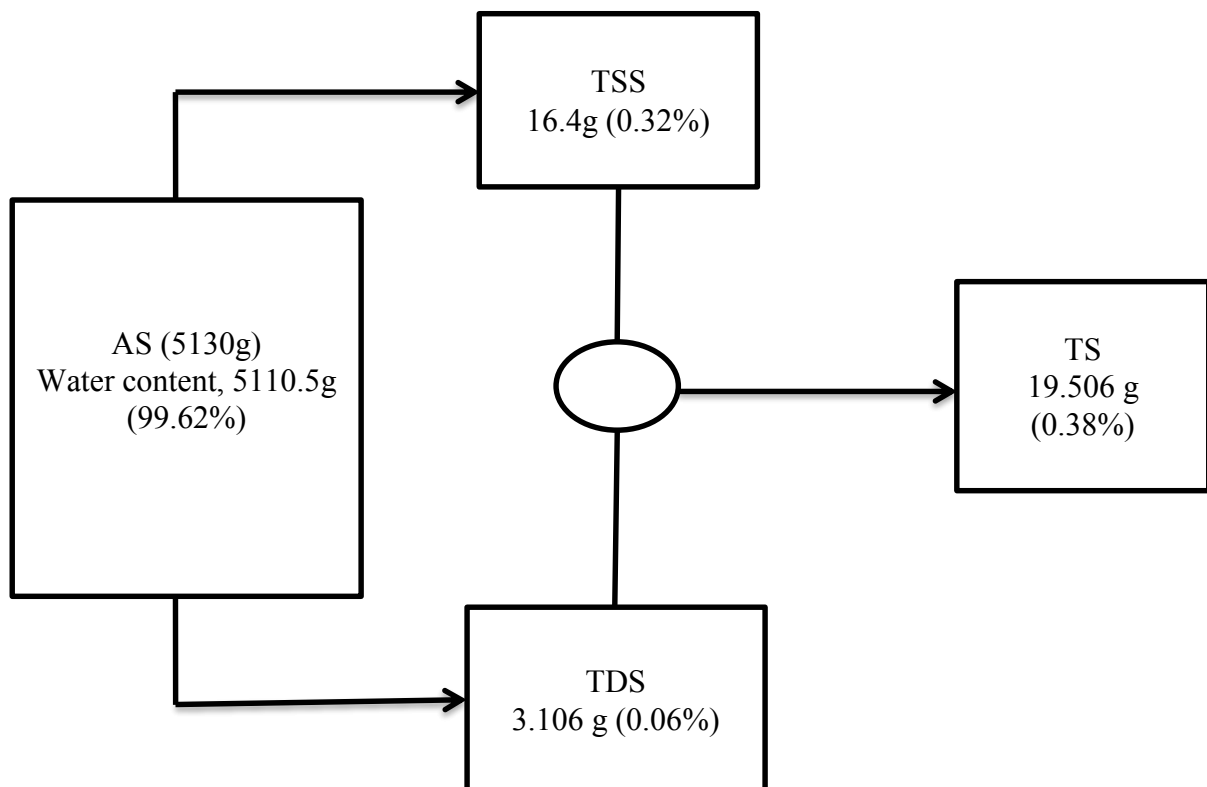


Figure 3-1: example of biomass partitioning in activated sludge from Finham STW (batch 1)

might be due to high proportion of suspended biological solid in the activated sludge which can easily be separated by filtration (Templeton and Butler, 2011). The results were further

expressed in terms of biomass concentration in order to demonstrate the amount that can be obtained from a litre of activated sludge (Table 3-1). Based on two STW sampled, it was found

Table 3-1: solid biomass concentrations partitions in the activated sludge

S/N	Source of the activated sludge	Batch of activated sludge from the industry	Activated sludge TS ¹ (g/L) (data from industry)	Activated sludge Concentrations		
				TSS (g/L)	TDS (g/L)	TS (g/L)
1	Finham STW	1	~4.00	3.20	0.61	3.80
2	Finham STW	2	~3.00	2.85	0.32	3.17
3	Finham STW	3	*	2.92	0.29	3.21
4	Minworth STW	1	~3.00	-	-	2.81
5	Minworth STW	2	*	2.14	0.29	2.51

(STW = sewage treatment works, 1, 2, 3 are batches, and TS¹ = activated sludge data collected from industry (WatStech Ltd, United Kingdom), S/N = serial number, * = data not available and - = not determined)

that the maximum and minimum concentration of TS from Finham STW were 3.80 and 3.17 g/L and those from Minworths STW were 2.81 and 2.51 g/L, respectively. These concentrations are corroborated with the data obtained from the industry which provided a range of TS in activated sludge, from Finham STW as 3.0 – 4.0 g/L and that from Minworth STW as 2.0 – 3.0 g/L (WatStech, 2015). Templeton and Butler (2011) further confirms these specifications as they state that the activated sludge process has a concentration of 2.0 – 8.0 g/L. With our findings, we reasoned that for a sewage works like Minworth that receives an average flow of approximately 540,000 m³/day, out of which 1300 m³ of activated sludge slurry can be produced per day. The activated sludge biomass equivalent would be in the range of 2000 to 3000 mg/L (Severn Trent Water, 2015). Thus, a total of 2600kg/day to 3900 kg/day of solid activated sludge biomass can be available for lipid extraction.

3.2.1.2 Investigating the potential effect of seasonal and regional variation in activated sludge biomass content

This was carried out in order to assess if seasonal and regional variation had any impacts on the quantity of activated sludge biomass recovered from the two STW. During the period of study, activated sludge samples were collected from STW, located at two different regions, in the United Kingdom, at various seasons in the year. The biomass was obtained as described in Section 2.2.1.1, that is, through filtration, followed by drying processes. The results are presented in Figure 3-2 as the percentage TS and it showed that there was a variation in biomass obtained from the same region at different season and across the regions. The maximum biomass from Finham STW was 0.38% and that from Minworth 0.28%. Although, the two sewage works receive their wastewater from predominantly domestic and industrial waste with less amount of agricultural waste, the TS differed. The reason for this might be due to variation in season and region, as shown in Figure 3-2. The catchment area contributes to this variation, Finham STW serves a population of 450, 000 people and Minworth 1.7 million people (Severn Trent Water, 2015). The findings herein are in agreement with the positions of United-Nations (2003) and Weiner and Matthew (2003) in which they stated among other factors that the flow of wastewater to the STW varies with community size, degree of industrialization, season, day and hour of sampling. Similarly, Revellame et al. (2012a) and Nguyen et al. (2013) affirm that lipid yields varies with time in which the activated sludge was sampled and the source of wastewater.

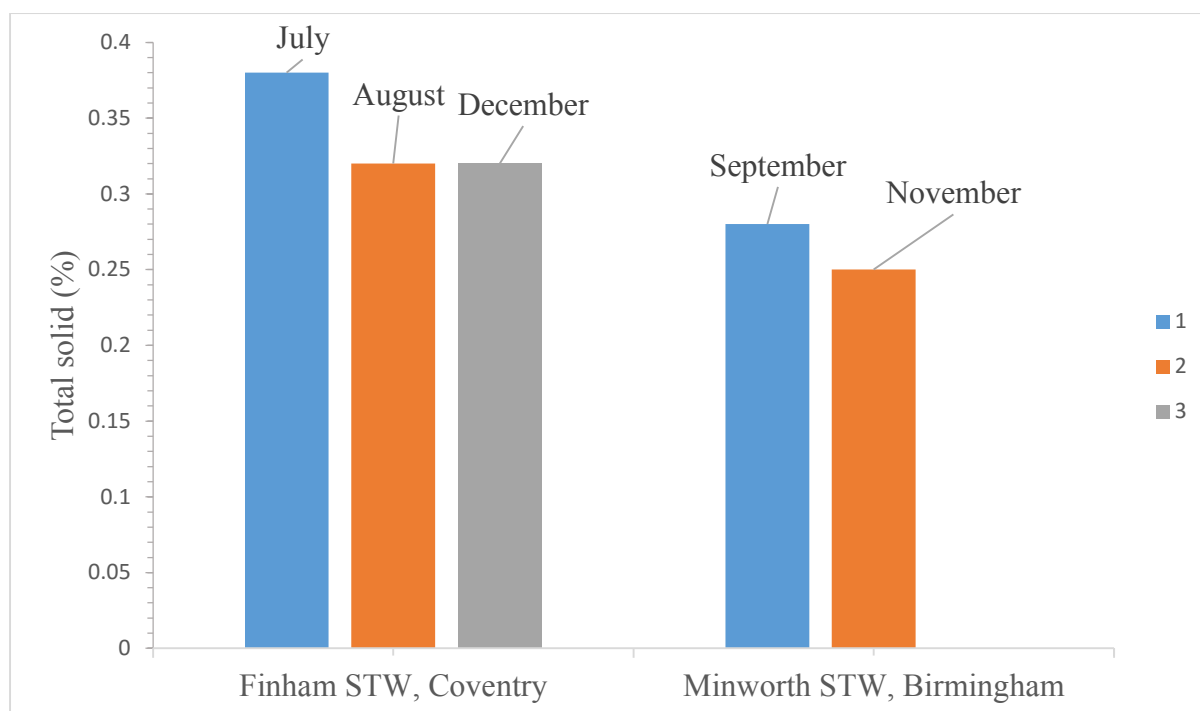


Figure 3-2: total solid obtained from activated sludge biomass as a function of Sewage Treatment Works and date of samples collection (STW = sewage treatment works)

3.2.1.3 Determining the moisture content in the activated sludge biomass

The amount of water remaining in the total activated sludge biomass after drying was determined in order to assess its impact on the lipid content of the activated sludge. This was done using 1g of dried activated sludge biomass, temperature 105°C and drying time 24 h, according to the method described in Section 2.2.1.1.2. The results are expressed as percentage moisture content (Table 3-2). The moisture content in the activated sludge from Finham STW was 1 - 11.84% and that from Minworth STW was 6.36 - 11.2%. The presence of water in the activated sludge biomass obtained from different seasons, posed no adverse effect on the lipid content determined using the same extraction method, see Section 3.2.2 (Table 3-3). For instance, 7.40 (wt/wt) % and 7.31 (wt/wt) % of lipid yields were obtained from activated sludge biomass containing 11.2 % and 7.68% from Minworth STW, batches (1 and 2). This might be because, the activated sludge biomass was granulated, thereby, increasing the surface area of particles bearing lipid to interact with the solvents.

Table 3-2: moisture content of the activated sludge

S/N	Source of the Surplus activated sludge	Batch of the activated sludge biomass	Amount of activated sludge biomass (mg)	% moisture content
1	Finham STW	1	19.51	1.00
2	Finham STW	2	68.03	11.84
3	Finham STW	3	72.38	6.36
4	Minworth STW	1	32.42	11.20
5	Minworth STW	2	48.62	7.68

(STW = sewage treatment works and 1, 2, 3 are batches of activated sludge collected)

3.2.2 Lipid extraction from activated sludge using organic solvent

Lipid was extracted from activated sludge using Soxhlet apparatus or Folch method, in order to evaluate the potential of the utility of the biomass as a feedstock for bioenergy (biodiesel and renewable diesel) and oleochemicals production. The results are expected to be used as a basis for evaluating the performance of the subcritical water mediated extraction, which would be carried out in order to improve lipid yield. The organic solvent: methanol 175 mL was used to extract lipid from 5g of activated sludge biomass in Soxhlet apparatus (Section 2.3.1.1). While chloroform/methanol (2/1, v/v) 20 mL was employed to extract lipid from 1g of biomass using Folch et al. (1957) method (Section 2.3.1.2). The lipid recovered from both methods were compared. Lipid was further extracted from activated sludge TDS from Finham STW to recover total lipid and to compare with that obtained from TSS using Folch et al. (1957) in order to determine which part had a greater lipid concentration. The results of lipid extracted from TSS and TDS are presented in Table 3-3 and they show that Soxhlet apparatus gave higher lipid yield 3.97 (wt/wt) % from Finham STW biomass compared to Folch method. According to Zhu et al. (2012), the lipid yield from the same samples depends generally on the nature of

the organic solvent. Polar unlike non-polar solvents extract more lipid (Manirakiza et al., 2001, Zhu et al., 2014). Thus, the higher lipid yield obtained from Soxhlet method can be attributed to the methanol used which is more polar than the chloroform/methanol (2:1, v/v) employed with the Folch method, apart from its natural ability to extract greater amount of sample mass compared to other extraction methods (Luque de Castro and Garcia-Ayuso, 1998). Although,

Table 3-3: results of the lipid extraction from the activated sludge using organic solvent

S/N	Method of extraction	Batch of activated sludge	STW	lipid yield (wt/wt) %		
				TSS	TDS	TS
1	Soxhlet	1	Finham	3.97 ± **	*	3.97
2	Folch	1	Finham	2.67 ± 0.67	0.09	2.76
3	Folch	2	Finham	3.33 ± 0.24	0.59	3.92
4	Folch	1	Minworth	*	*	7.40 ± 0.58
5	Folch	2	Minworth	*	*	7.31 ± ***

(* = not determined as the entire slurry was dried due to slow filtration, ** = no replicate due to insufficient biomass, *** = no replicate as the organic phase post extraction was pooled together to ensure homogeneity in lipid obtained after drying, TSS = total suspended solid, TDS = total dissolved solid, TS = total solid and STW = sewage treatment works). The amount of activated sludge (TSS) used in Soxhlet apparatus was 5g. The amount of TSS and TDS, and TS used in the Folch method were 1g and 5g, respectively.

Soxhlet gave a higher yield of lipid than the Folch method, it was found to require large amount of the activated sludge, long extraction time and high volume of organic solvent (Luque de Castro and Garcia-Ayuso, 1998, Naude et al., 1998). While Folch method required less extraction time, less amount of activated sludge biomass and less volume of the organic solvent. The chloroform-methanol used with the Folch method is a good solvent combination according to Smedes and Askland (1999) for determining the total lipid (neutral and polar lipids). This solvent combination ensured that cell membrane lipids such as phospholipids and cholesterol were extracted using methanol while neutral lipids such as triglycerides and waxes

by chloroform (Ferraz et al., 2004). The capacity of the Folch method to extract total lipids from the activated sludge gave it preference in addition to the other mentioned advantages over the Soxhlet.

Furthermore, the lipid yields obtained from the total dissolved solid (TDS) and total suspended solid (TSS) differed considerably, from same batch of activated sludge obtained from Finham STW (batches 1), in the same seasons (summer). The lipid yield obtained from the TDS was 0.09% as against 2.67 (wt/wt) % from TSS using Folch method from activated sludge obtained in July. While 0.59 (wt/wt) % and 3.33 (wt/wt) % of lipid yields were obtained from TDS and TSS, respectively, using Folch on the activated sludge obtained in August. This disparity was expected, since the activated sludge possessed biological solid biomass, which readily settled and was recovered as TSS, and TDS contains colloidal, inorganic materials and little amount of organic materials which either escaped during filtration or dissolved under prolonged storage, (Templeton and Butler, 2011). It was demonstrated in Table 3-3 that the maximum lipid yield from Minworth STW 7.40 (wt/wt) % was greater than that of Finham STW 3.97 (wt/wt) %. Similarly, Table 3-4 shows that more lipid was extracted per litre from Minworth STW than from Finham STW. Thus, the former offers a greater potential for bioenergy (biodiesel and renewable) and oleochemicals production than the latter STW. The reasons for this disparity could be attributed to difference in catchment area, degree of industrialization, season, day and hour of sampling the activated sludge slurry (United-Nations, 2003, Weiner and Matthew, 2003).

Table 3-4: concentration of lipid in activated sludge biomass

S/N	Batch of activated sludge	STW	biomass concentration (mg/L)	Concentration of lipid in activated sludge biomass (mg/L)		
				TSS	TDS	TS
1	1	Finham	3804.6	126.0 ± *	3.4	129.4
2	2	Finham	3174.1	105.7 ± 7.6	18.7	124.4
3	1	Minworth	2805.9	*	*	207.6 ± 16.3
4	2	Minworth	2507.2	*	*	183.27 ± **

(* = not determined as the entire slurry was dried due to slow filtration, **= no replicate due to insufficient biomass and *** lipid concentration obtained from two methods, one of which had no replicate, annotations of Table 3-3)

Comparing the maximum lipid yield 7.4 (wt/wt) % obtained with those in literature gave a clear dependence on source of biomass, solvent, method of extraction and operating conditions, as can be seen from the works done by the following investigators. The study carried out by Siddiquee and Rohani (2011) show that a maximum lipid yield (dry sludge basis) of 10.04 (wt/wt) % and 3.04 (wt/wt) % were obtained from activated sludge according to a modified Dufreche et al. (2007) and Boocock et al. (1992) methods, using methanol and hexane, respectively. Dufreche et al. (2007) used a 200 series accelerated solvent extraction at 100°C for 1 h to obtain a maximum lipid yield of 27.43 (wt/wt)% using hexane/methanol/acetone (60/20/20, v/v/v) and minimum of 1.94 (wt/wt)% employing 100% hexane from activated sludge. Revellame et al. (2012a) extracted a lipid yield of 6 – 16% by Bligh & Dyer extraction (BDE) using methanol, chloroform and water from three batches of activated sludge biomass obtained in different months of the year. Huynh et al. (2010b) used Soxhlet extraction with hexane to obtain a neutral lipid yield of 2.10 (wt/wt) %. Olkiewicz et al. (2012) carried out Soxhlet extraction on activated sludge using hexane and they obtained 9.3 ± 1.3 (wt/wt) % lipid yield. Nguyen et al. (2013) worked on Soxhlet extraction of dewatered activated sludge from four different STW and obtained a minimum of 1.67 ± 0.19 (wt/wt) % and maximum of 66.64

± 1.21 (wt/wt) % lipid yields. The lipid yields were obtained from activated sludge with water contents of 91.39 ± 0.20 (wt %) and ~ 80 (wt %).

3.2.3 Characterization of lipid extract from activated sludge biomass

The results of the acid value, lipid composition and fatty acid methyl esters of the lipid extract are presented.

3.2.3.1 Determining the acid value of the lipid extract

This was determined in order to be guided on the choice of catalyst for fatty acid methyl ester (FAME) production and on the expected acidic property of biodiesel, according to AOAC (2005) Modified titrimetric method. This method was developed from AOAC (2005) and validated using Sunflower oil. The acid value was obtained by titrating 40 mg of oil or lipid extract (from Finham STW, batch 2) with 1.42 mM NaOH (Section 2.8.1). The results are expressed as %FFA (free fatty acid (oleic acid)) and acid value (Table 3-5). The acid value

Table 3-5: acid values and percent oleic acid in the sunflower oil and lipid extract

S/N	Lipid/oil	Method of determination	% FFA (oleic acid) in the lipids	Acid value (%)
1	Sunflower oil	AOAC (2005)	2.3 ± 0.58	4.6 ± 1.15
2	Sunflower oil	AOAC (2005) modified	2.5 ± 0.28	5.0 ± 0.56
3	Lipid extract	AOAC (2005) modified	20.5 ± 0.26	40.9 ± 0.51

(The acid value / %FFA of lipid extract from Minworth was not determined due to limited amount of biomass, AOAC = Association of Official Analytical Chemists)

from refined sunflower oil using AOAC (2005) and AOAC (2005) Modified are in close agreement and hence, the later method was used with the lipid extract. The high value of the % FFA or acid value obtained from the sunflower oil was probably due to the methods being

optimised for crude oil analysis. As shown in Table 3-5, the % FFA of the lipid extract was 20.5 ± 0.26 %, and that of acid value was 40.9%, this suggests that an acidic catalyst is preferred for FAME production and that the biodiesel would be acidic. The high value of the %FFA is also an indication that the prospect of producing biodiesel from the lipid extract would be difficult using an alkaline catalyst (Van Gerpen et al., 2004). It was observed from the results of the lipid extract analysis that the most prominent fatty acid was palmitic acid. Thus, if the %FFA or acid value was calculated as a function of the palmitic acid, a reduction of 12.2% or 9.3%, respectively would be achieved (Olkiewicz et al., 2014). Unlike, the sunflower oil whose high %FFA value was attributed to the method which was originally meant to be used with crude oil, that of the lipid extract was partly due to the sources of the activated sludge. The activated sludge was derived from both industrial and domestic waste water sources which were known to contain lipids such as spent or used oil, greases, fats or long-chain fatty acid (Becker et al., 1999, Henze, 1992, Quemeneur and Marty, 1994, Raunkjaer et al., 1994). These lipids, especially FFA are not easily decomposed compared to sugars and amino acid, and therefore, tend to accumulate in the secondary waste water treatment line, resulting to the increased %FFA obtained (Chipasa and Mdrzycka, 2008). Also, the spent oil or used oil according to Marchetti and Errazu (2008) contains between 3% and 40% FFA, which thus, validated the results obtained from the lipid extract. Although, the %FFA of the lipid extract may be high, it is still a better substrate for the production of biodiesel using an alkaline catalyst compared to the 60 and 36% obtained by Boocock et al. (1992) and Huynh et al. (2010a), respectively. The high %FFA or acid value could have resulted also from the hydrolysis of triacylglycerol by extracellular enzymes such as lipase or breaking of the long chain in the lipid extract due to thermal requirement for the recovering of the activated sludge and extraction of lipids (Dueholm et al., 2001, Hwu et al., 1998, Kargbo, 2010, Karmakar et al., 2010). The high % FFA or acid value obtained was an indication that for a potential use of the lipid extract in

the biodiesel production, an acid catalyst should be considered. While the use of a basic catalyst in the production of biodiesel from a high % FFA lipid extract would result in soap formation which would invariably lead to difficulty in separation and low FAME yield (Kargbo, 2010). The biodiesel produced from a high % FFA lipid extract might cause a corrosion in the fuel supply system and in the internal combustion engine (Atabani et al., 2013).

3.2.3.2 Determining the lipid composition in activated sludge

It was necessary to assess the lipid composition in activated sludge, so as to determine the types of oleochemicals that could be obtained, their potential as substrates for bioenergy (biodiesel and renewable diesel) production and impact on its qualities. This was done using high performance thin layer chromatography (HPTLC), see Sections 2.9.1.1.1, 2.9.1.1.2 and 3.2.3.2.1 and gas chromatography (see Sections 2.8.3 and 3.2.3.2.2). The identification of the lipid classes in the lipid extract was also done using thin layer chromatography (TLC), see the Appendix.

3.2.3.2.1 Analysing the lipid composition in activated sludge

(A) Qualitative analysis of neutral lipids and phospholipids in the lipid extract from the activated sludge using HPTLC

The presence of lipid compounds in the lipid extract was investigated according to the method described in Section 2.9.1.1.1. The HPTLC chromatographic process was carried out using two 20 x 10 cm glass HPTLC plates which were developed separately. This was necessary, as cholesterol and dioleoyl glycerol lipid standards used to identify sterol and acylglycerol showed the same retention factor, thereby, making it difficult to separate them on the same plate. Other lipid standards: oleic acid, cetyl palmitate and monoacylglycerol (MAG) were used to identify free fatty acid, wax ester and acylglycerol, respectively. The amount of the lipid

extracted loaded on the plates was 25µg. While the minimum amount of the lipid standards loaded was 0.5µg and the maximum amount was 10µg. The plates were developed using petroleum ether/diethyl ether/acetic acid (80/20/1, v/v/v) which was the optimum eluent solvent obtained using TLC (see Appendices 8.3.2 and 8.3.3). The migration distance covered by the elution process was 85mm. The destructive method of lipids detection was used to identify the lipids using charring conditions of temperature and time of 170°C and 5min respectively (Appendices 8.2.1.1.1 (3) and 8.3.1 (iii)). The results show the presence of the following neutral lipid classes sterol, wax ester, acylglycerol and free fatty acid (Figures 3-3 and 3-4), see Section 3.3.3.2.1. These were corroborated with the lipids classes identified using TLC, see Appendices 8.3.2 and 8.3.3. These neutral lipid classes are discussed in Section 3.2.3.2.1 (B) below.

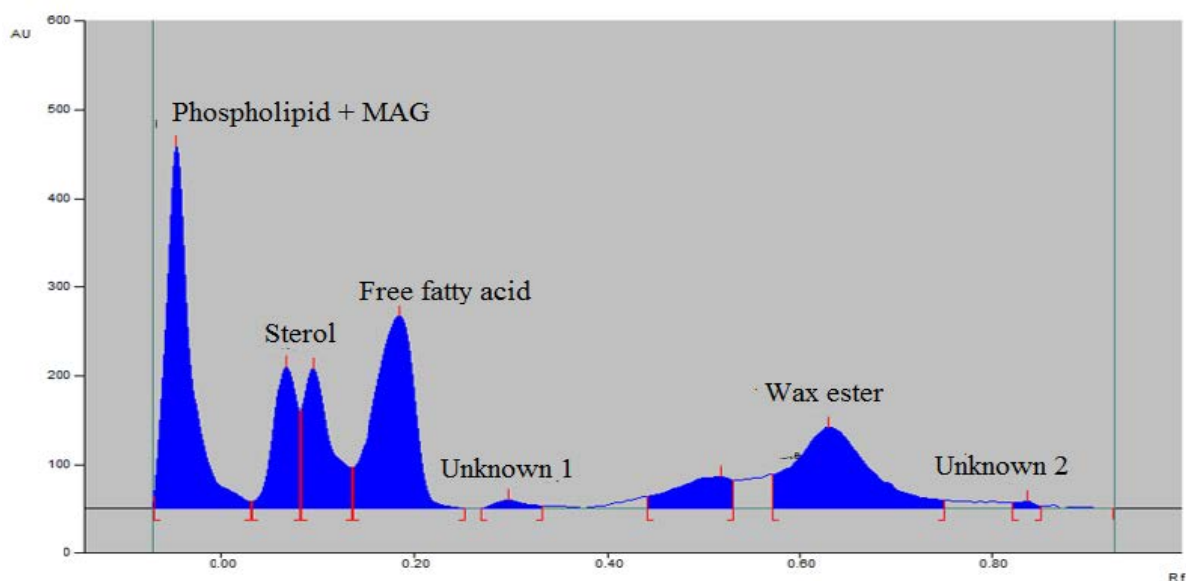


Figure 3-3: neutral lipids identified in the lipids extract from the activated sludge (Finham STW, batch 2). Plate developed using petroleum ether/diethyl ether/acetic acid (80/20/1, v/v/v) and detected at 170°C after 5min

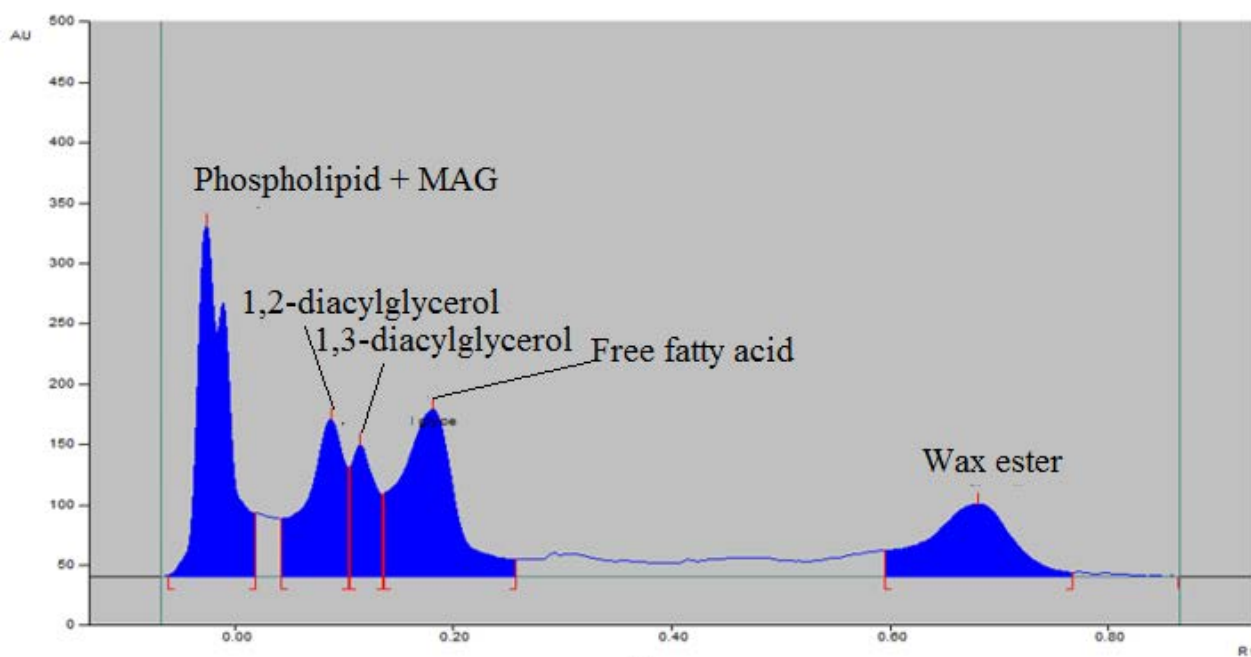


Figure 3-4: neutral lipids classes identified in the lipids extract from the activated sludge (Finham STW, batch 2) (contd). Plate developed using petroleum ether/diethyl ether/acetic acid (80/20/1, v/v/v) and detected at 170°C after 5 min

The phospholipids composition in the lipid extract was assessed according to the method described in Section 2.9.1.1.1. The lipid standards used were L- α -phosphatidylcholine (PC) and phospholipids standard mix comprising L- α -lysophosphatidylcholine (LPC) (300.4, wt. conc.), L- α -phosphatidylcholine from soybean (PC) (1500, wt. conc.), L- α -phosphatidylethanolamine (PE) (1200, wt. conc.) and L- α -phosphatidylinositol (PI) (899.8, wt. conc.). The plate was developed using chloroform/methanol/28% ammonia (65/25/5, v/v/v) (Vitiello and Zanette, 1978). The results show the presence of LPC, PC, PE and PI in the lipid extract, although, the amount of LPC under the current conditions was quite small, due to its low concentration relative to other phospholipids in the standard mix (Figure 3-5). The LPC was identified when the resolution of the lipid bands was increased, but the effect of this was a very dark background which made other phospholipids bands invisible. These results are in agreement with those obtained using TLC, see Appendix 8.3.3.

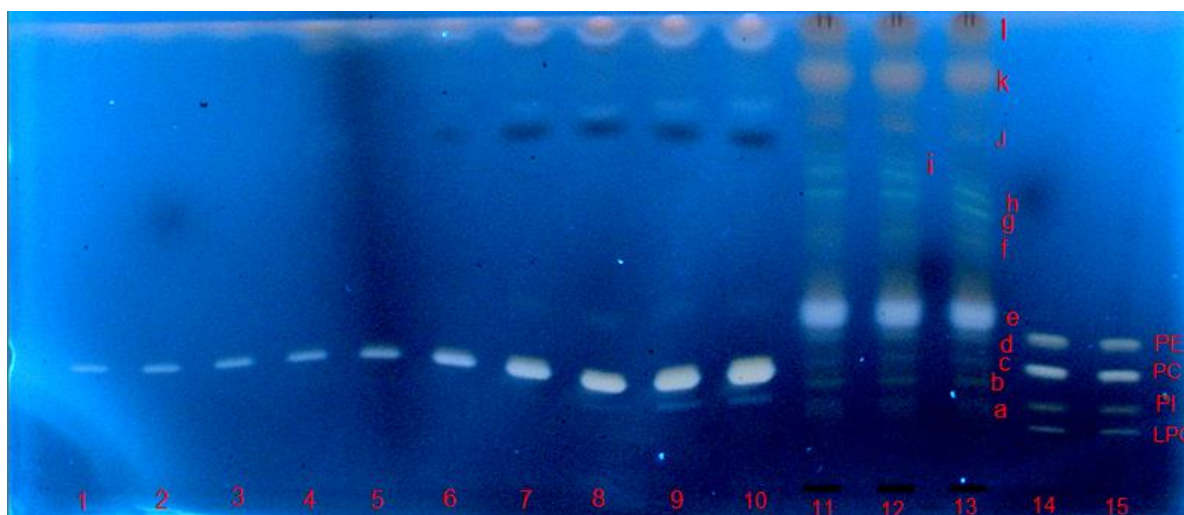


Figure 3-5: a developed HPTLC plate for the identification of phospholipids from the lipids extract obtained from Finham STW (batch 2). Tracks 1 – 10 represented different loads of PC, the lipid-extracts were applied at tracks 11 – 13 while the phospholipids standard mix was loaded on tracks 14 and 15. The lipids identified were a (PI), c (PC), d (PE) while LPC was not visible at the resolution used to obtain the image. Also, c, e, f, g, h, I, j and were unidentified phospholipids while I was part of the neutral lipids eluted to the solvent front by the eluent. Solvent system: chloroform/methanol/28% ammonia (65/25/5, v/v/v)

(B) Quantitative analysis of lipid extract from the activated sludge using HPTLC

In order to determine the lipid classes and amount of neutral and polar lipids (phospholipids) identified in activated sludge (Finham STW, batch 2), which will be available for bioenergy (biodiesel and renewable diesel) production, the lipid extract was analysed using high performance thin layer chromatography (HPTLC), as described in Section 2.9.1.1.2. The concentration of lipid extract applied on the plate was 25 µg/ µL and that of the lipid standards were 100 ng / µL, respectively (Table 2-10). The solvent system used for quantifying neutral lipid was petroleum ether/diethyl ether/acetic acid (80/20/1, v/v/v). The results are presented in Figure 3-6 and they show that 1, 2-acyl glycerol was the predominant lipid in the activated sludge. The neutral lipid classes obtained are discussed below.

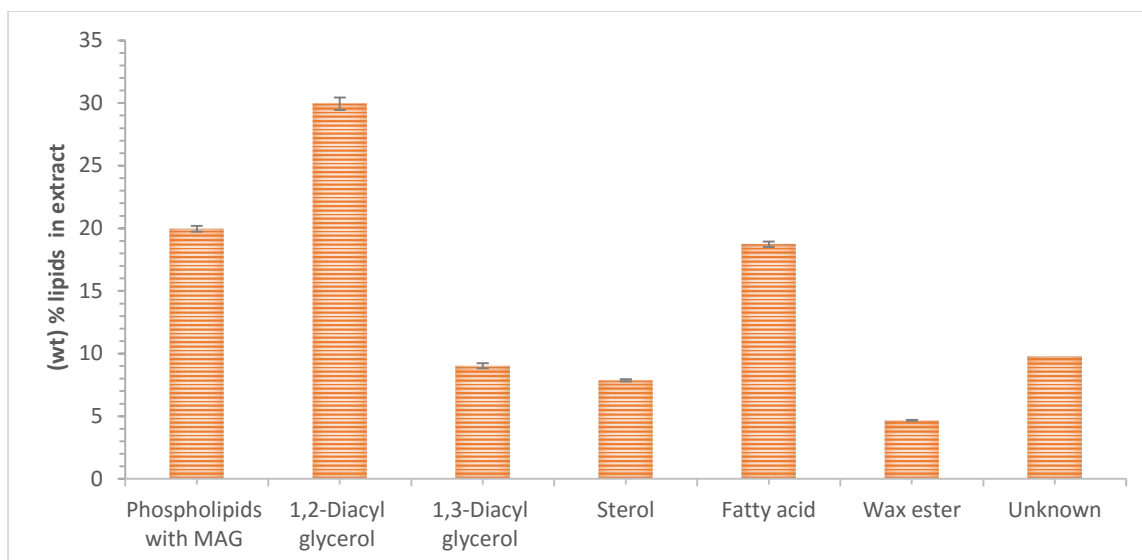


Figure 3-6: composition of lipid in the lipid extract from the Finham STW (batch 2) activated sludge ($n = 3$), obtained by HPTLC using petroleum ether/diethyl ether/acetic acid (80/20/1, v/v/v) as the system (phospholipids showed close retention factor with MAG (monoacylglycerol). MAG, 1,2-dicylglycerol and 1,3-diacylglycerol were used to identify the acylglycerol lipid class.

Waxes – these are found in the animal and microbial tissues, with wax ester as the most predominant (Christie, 2011). Wax esters have been reported to be present in wastewater (Quemeneur and Marty, 1994). The HPTLC analyses show the presence of wax esters in the activated sludge (Finham STW, batch 2). This is corroborated by the findings of Huynh et al. (2011) and Revellame et al. (2012a). As shown in Figure 3-6, wax esters represents 4.67 (wt. %) of the lipid extract from activated sludge (Finham STW, batch 2). Wax esters are used for the production of cosmetics, lubricants, polish, waterproof and surface coating (Huynh et al., 2011).

(ii). **Acylglycerol (acylglyceride)** – These include triacylglyceride, diacylglyceride and monoacylglyceride. Triacylglycerides are esters of fatty acids and glycerol which are found in fats and oils of plants and animal origin (Christie, 2003, Sahena et al., 2009). Diacylglyceride and monoacylglyceride are present in trace quantities in both fresh plant and animal tissues,

but, can be biosynthesized from the hydrolysis of triacylglyceride and phospholipids (Christie, 2011). As shown in Figure 3-6, the lipid extract contained 29.95 (wt. %) of 1,2-diacylglyceride, 9.03 (wt. %) of 1, 3- diacylglyceride and small amount of monoacylglycerol, with diacylglyceride constituting the largest proportion. There was no triacylglyceride in the lipid extract, as it is believed to have been hydrolysed to diacylglyceride and monoacylglyceride (Revellame et al., 2012a). Acylglycerides are applied in the production of soaps, detergents, plastic and renewable energy (Revellame et al., 2012a).

(iii). Sterols – these are found in the tissues of animals, the predominant of which is cholesterol (Christie, 2003). As shown in Figure 3-6, the lipid extract from activated sludge contains 7.86 (wt. %) of cholesterol. Other researchers Revellame et al. (2012a) and Quemeneur and Marty (1994) have also demonstrated that activated sludge and wastewater in general, contain sterols such as cholesterol, stigmasterol and coprostanol. The sterols are used in the steroids synthesis, drug production, as surfactant and for renewable energy production (Revellame et al., 2012a).

(iv). Free fatty acid - These are fatty acids that are not attached to any molecules (Christie, 2003). They are not easily degraded and thus, accumulate during the biological treatment of wastewater (Chipasa and Mdrzycka, 2008, Novak and Kraus, 1973, Pei and Gaudy, 1971). The free fatty acid content of the lipid extract has been discussed in Section 3.3.3.1. Like the glycerides, they are used for soap making, detergent and renewable energy production (Revellame et al., 2012a).

The phospholipids: PE, PC and PI in the lipid extract from activated sludge (Finham STW, batch 2) were quantified to determine the amount that is available for bioenergy (biodiesel and renewable diesel) and oleochemical production using the method presented in Section 2.9.2.1.2. The solvent system used were chloroform/methanol/28% ammonia (65/25/5, v/v/v).

The amount of lipid extract applied on the plate was 25 µg and that of the standards are presented in Table (2-10). As shown in Figure 3-7, the maximum phospholipid obtained from

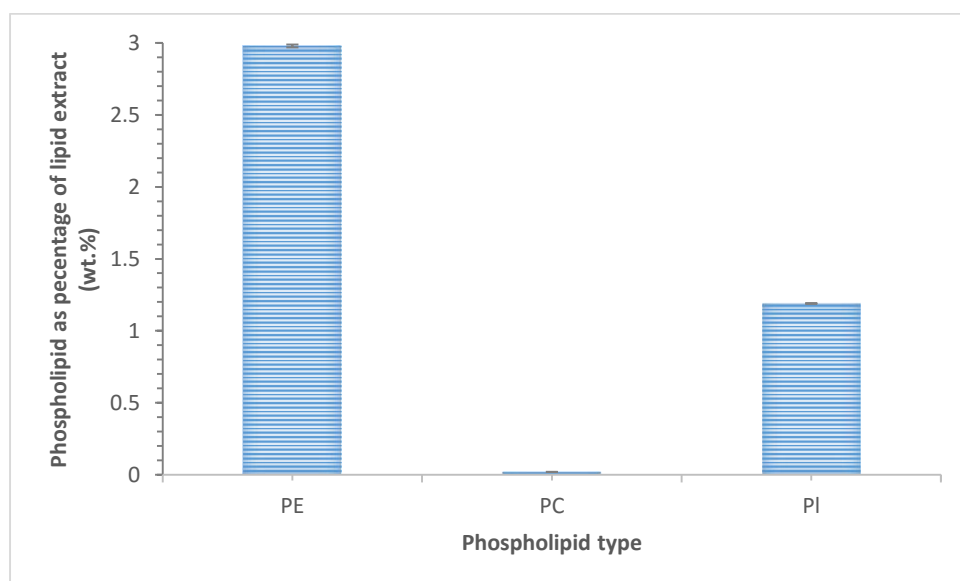


Figure 3-7: composition of phospholipids in lipid extract from Finham STW (batch 2) activated sludge (n = 3) obtained by HPTLC using chloroform/methanol/28% ammonia (65/25/5, v/v/v)

the lipid extract was phosphatidylethanolamine 2.98 (wt. %) and it corresponded to 0.09 (wt. %, dry activated sludge). This maximum yield is in agreement with that obtained by Revellame et al. (2012a), in which they presented an optimum of 0.07 (wt. %, dry activated sludge) phospholipids. Generally, phospholipid is used as a feedstock for oleochemicals and in production of biofuel (Revellame et al., 2012a).

3.2.3.2.2 Profiling the fatty acid in the lipid extract from activated

In order to determine the amount of esterifiable substances in the lipid extract from activated sludge samples (Finham STW, batch 2), which can be converted to biodiesel, its fatty acid content was established, as described in Section 2.4. The lipid extracted from activated sludge and fatty standards were converted to methyl ester through hydrochloric acid (HCl) catalysed methanolysis reaction and the products were analysed using GC (Section 2.9.3.1). The fatty

acid standards of the FAMES identified in activated sludge were combined in order to obtain the profile. This profile of derivatized FAMES was validated using a grain fatty acid methyl ester mix. The results obtained are presented in Figure 3-8 and they illustrate a typical FAMES profile, where the principal fatty acids were palmitic acid (C16:0), palmitoleic acid (C16:1n9C), linoleic acid (C18:2n6C), oleic acid (C18:1n9C) and stearic acid (C18:0). Palmitic acid was found to be the most predominant fatty acid in the activated sludge (Finham STW, batch 2). The fatty acid profile obtained is in agreement with those presented in the literature by Mondala et al. (2009) and Revellame et al. (2010). As have been demonstrated in

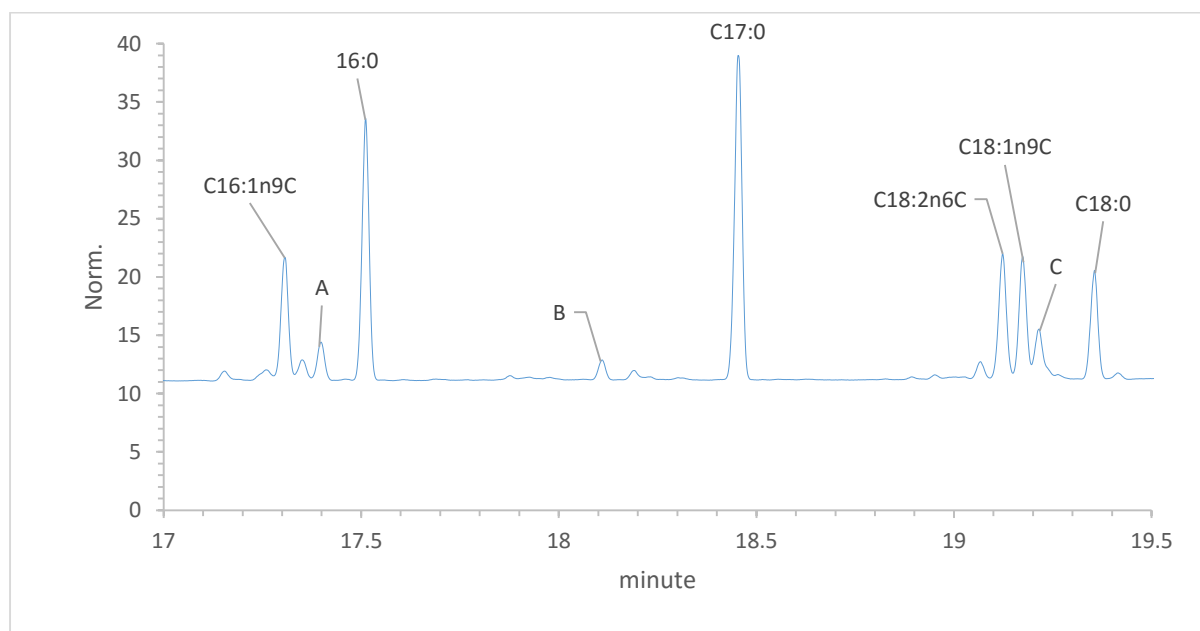


Figure 3-8: profiles of the FAMES obtained from the transesterification of the lipid-extract from the activated sludge (Finham STW, batch 2) using gas chromatography (Agilent Technologies 6850 Network GC system) (C16:1n9C = palmitoleic acid methyl ester, C16:0 = palmitic acid methyl ester, C17:0 = heptadecanoic acid methyl ester (internal standard), C18:2n6C = linoleic acid methyl ester, C18:1n9C = oleic acid methyl ester, C18:0 = stearic acid methyl ester and A, B, C = unknown)

Figure 3-8, the composition of the FAMES was between methyl esters of palmitic acid (C16:0) and stearic acid (C18:0), with C16:0 been the most prominent fatty acid. This result is corroborated by Siddiquee and Rohani (2011), Mondala et al. (2009), Dufreche et al. (2007), (Zhu et al., 2014) and Revellame et al. (2011). According to Huynh et al. (2010a), most methyl

esters of palmitic acid and stearic acid emanates from vegetable oils and fats, these are known to be the characteristics of the activated sludge from domestic and industrial waste origin. Thus, since the activated sludge used in this work was derived from domestic and industrial wastes, the fatty acid composition was in agreement with Huynh et al. (2010a).

3.2.3.2.3 Evaluating the effect of operating parameters on FAME production

(1) (a). Impact of residence time on FAMEs production

The effect of residence time on the methanolysis of lipid extract was assessed using the method described in Section 2.4, in order to ensure that maximum FAMEs production were achieved. The methanolysis was carried out at residence times 5 min, 30 min, 1 h, 12 h and 24 h and catalysed by HCl. The resulting FAMEs were resolved using GC, as described in Section 2.9.3 and the results are illustrated in Figures 3-9. The results show that the appearance of the fatty acids in terms of the order of magnitude was palmitic acid, palmitoleic acid, oleic acid, stearic acid and linoleic acid per residence time and that 1 h was the optimum residence time required to produce the highest amount of esters from the activated sludge. As shown in Figure 3-9, the reaction favoured the production of palmitic acid per residence time compared to others fatty acids.

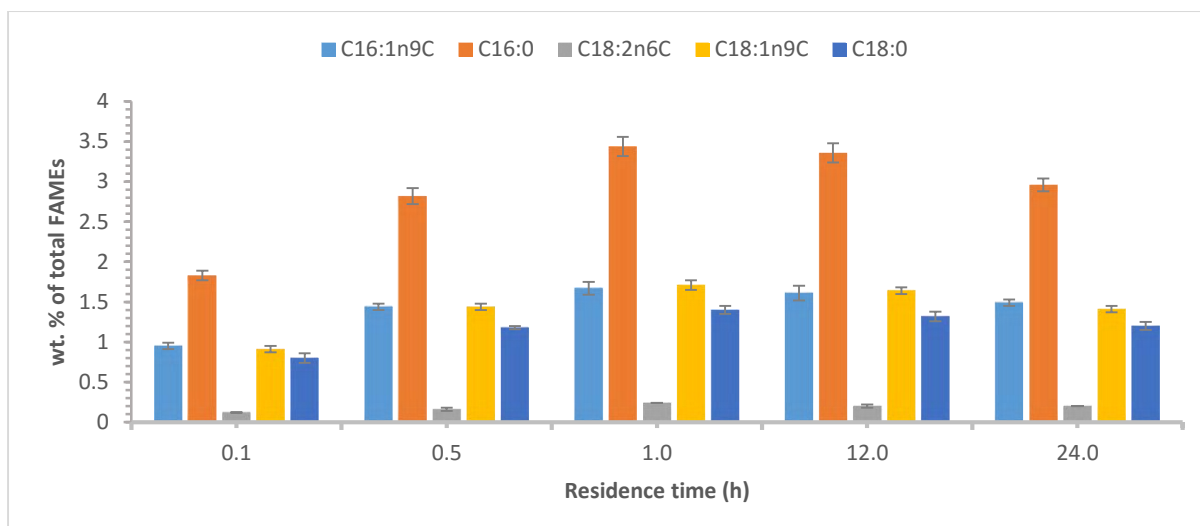


Figure 3-9: effects of residence time on the FAMES produced from the lipid extract from activated sludge (Finham STW, batch 2) (C16:1n9C = palmitoleic acid methyl ester, C16:0 = palmitic acid methyl ester, C18:2n6C = linoleic acid methyl ester, C18:1n9C = oleic acid methyl ester, C18:0 = stearic acid methyl ester)

The effects of residence times on yield of the FAMES produced with respect to dry activated sludge biomass showed that a minimum 0.16 (wt/wt) % was obtained at 5min (0.08 h) and maximum 0.41 (wt/wt) % at 1h (Figure 3-10). According to Ma and Hanna (1999), Mondala et al. (2009) and Issariyaku and Dalai (2014), the conversion rate of lipids and FAMES yield, increase with time. Thus, as shown in Figure 3-10, the yield of the FAMES increased rapidly between the time interval of 0.08 and 1 h, before it started to decline owing to the depletion of the acylglycerols in the lipid extract which led to a decrease in the reaction rate. The rapid increase in the yield of the FAMES between 0.08 and 1 h of the reaction, might have also resulted from the esterification of FFA in the lipid extract while the decline recorded after 12 h could be attributed to the thermal degradation at a long residence time (Siddiquee and Rohani, 2011). The composition of the FAMES shown in Figure 3-10 revealed increased yield at the optimum residence time of 1 h. Thus, the component FAME yields from the lipid extract were

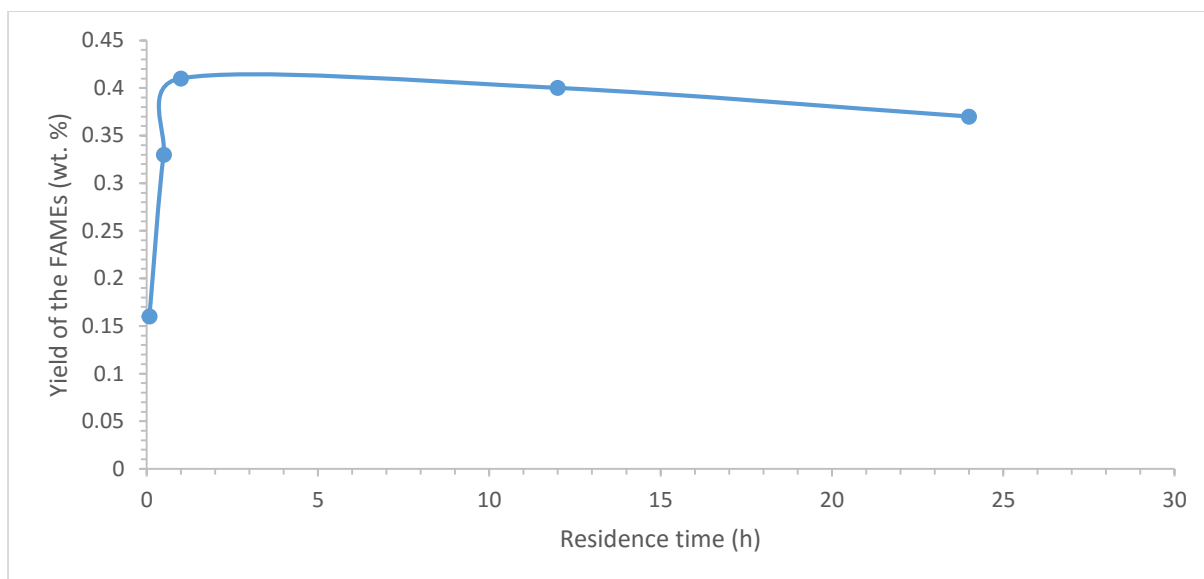


Figure 3-10: effects of residence time on yield of FAMES (with respect to dry activated sludge (Finham STW, batch 2)) produced from the HCl catalysed methanolysis of lipid extract

1.67, 3.44, 0.24, 1.71 and 1.4 (wt. %) for palmitoleic acid methyl ester (C16:1n9C), palmitic acid methyl ester (C16:0), linoleic acid methyl ester (C18:2n6C), oleic acid methyl ester (C18:1n9C) and stearic acid methyl ester (C18:0), respectively.

Furthermore, the FAMES yield was lower than those presented by Dufreche et al. (2007), Revellame et al. (2010), Mondala et al. (2009) and Pastore et al. (2013) which were 4.4 (wt. %), 4.79 (wt. %), 2.5 (wt. %) and 1.9 (wt. %), respectively. This might be attributed to the difference in the activated sludge source, methods of production and factors such as catalyst type, methanol-to-lipid (sludge) ratio, temperature, residence time, water and % free fatty acid contents (Huynh et al., 2012, Mondala et al., 2009, Siddiquee and Rohani, 2011). Although, the yield was not a surprise as the FAMES were produced from activated sludge from Finham STW which was known to contained a low lipid yield of 2.67 (wt/wt) % obtained using Folch method.

(1) (b). Investigating the impact of residence time on degradation of acylglycerol during the methanolysis of lipid extract from activated sludge

The effect of residence time on composition of acylglycerols (1, 2-diacylglycerol, 1, 3-diacylglycerol and monoacylglycerol) during methanolysis of lipid extract was evaluated, in order to monitor the reaction, as described in Section 2-4. This was done using HPTLC, as presented in Section 2.9.2.2, where the esters of the fatty acid produced during the reaction, eluted on the HPTLC plate into one single spot and was identified using the FAME standard mix. The chromatogram obtained is presented in Figures 3-11 and 3-12. The evaluation was conducted in triplicate with FAME produced at different residence times applied in duplicate on each HPTLC plate. The lipids identified were monoacylglycerol, 1, 2-diacylglycerol, 1, 3-diacylglycerol (acylglycerol) and wax ester. The results show that the free fatty acid was used completely before 5 min of reaction and there was a decline in acylglycerols as the reaction progressed from 5min to 72h (Figure 3-13). This was expected as that indicated that the lipid extract was consumed during the methanolysis reaction. The trend observed is in agreement with the results obtained by Darnoko (1999) and Ma and Hanna (1999). The initial increase in the amount of monoacylglycerol, 1, 2-diacylglycerol and 1, 3-diacylglycerol might have resulted from the hydrolysis of triacylglycerol and phosphatidylinositol (Christie, 2011, Pastore et al., 2013). It was also observed from Figure 3-13 that as the reaction progressed with time, 1, 2-diacylglycerol and 1, 3-diacylglycerol degraded to produce more monoacylglycerol which was successively converted to FAME and glycerol (Ma and Hanna, 1999).

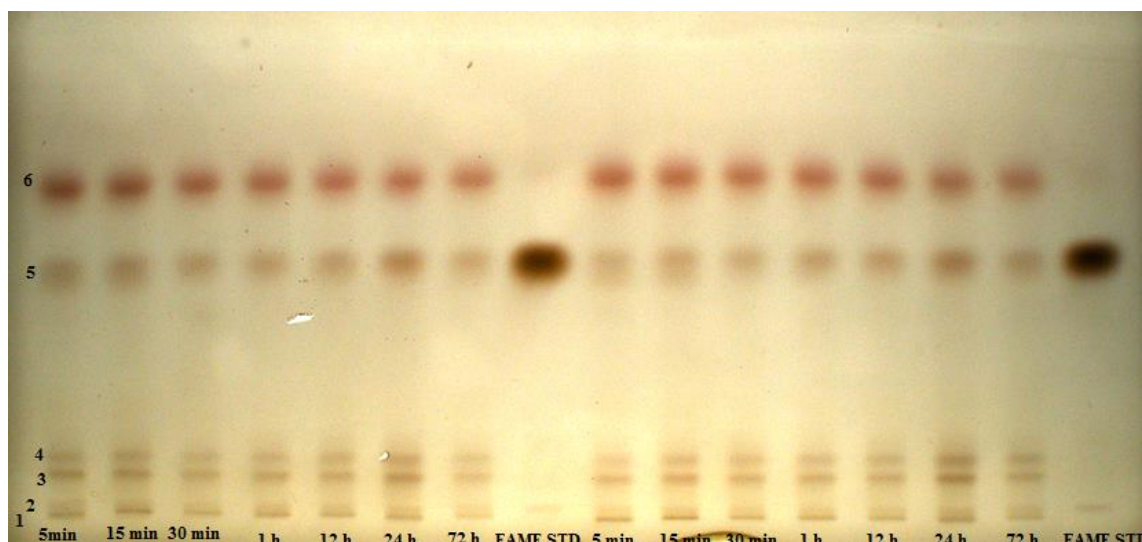


Figure 3-11: An example of the chromatograms of the products of the methanolysis of lipid extract using HCl as a catalyst (bands: 1= phospholipid, 2 = monoacylglycerol, 3 = 1, 2-diacylglycerol, 4 = 1, 3-diacylglycerol, 5 = FAME, 6 = cetyl palmitate). FAMEs samples were loaded at lanes 5min, 15min, 30min, 1h, 12h, 24h and 72h and the analysis was done in duplicate on the plate. The lipid extract was obtained from dry activated sludge (Finham STW, batch 2).

The lipids identified: monoacylglycerol, 1, 2-diacylglycerol, 1, 3-diacylglycerol and cetyl palmitate were quantified using HPTLC, as described in Section 2.11.2, in order to assess the degradation of acylglycerols as the reaction progressed with time, see Figures 3-12 and 3-13.

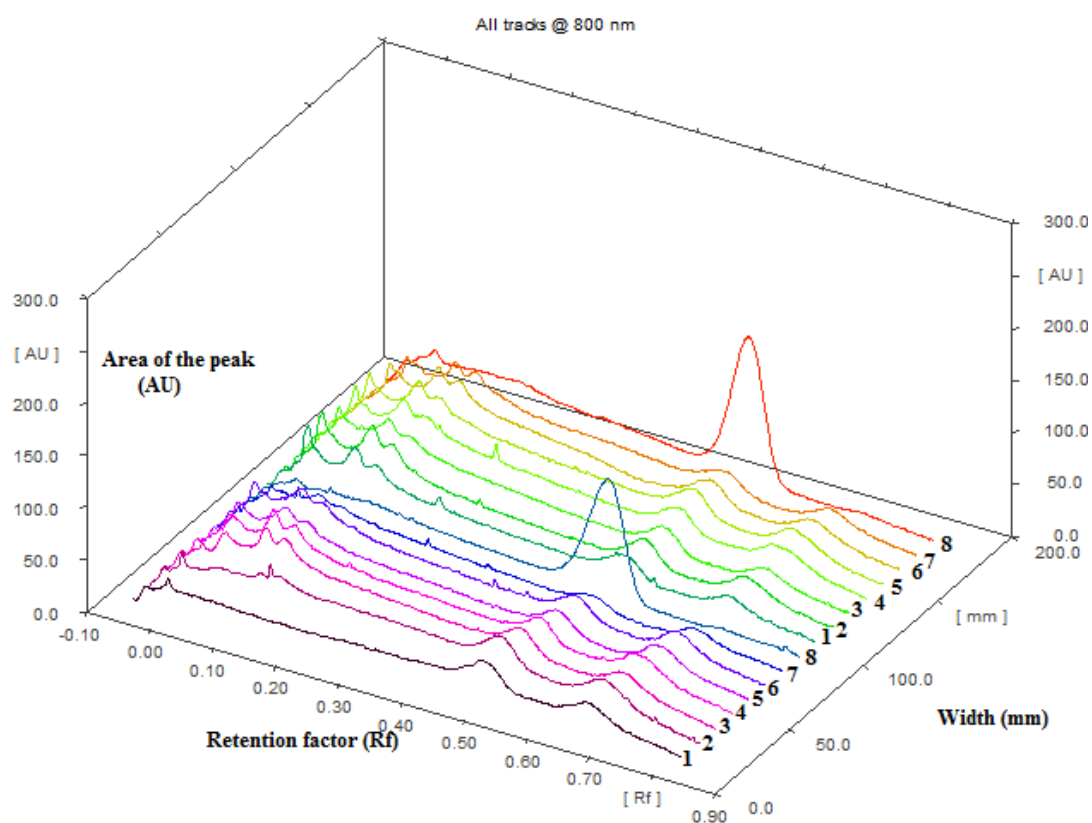


Figure 3-12: an example of a 3D plot of the chromatograms obtained from methanolysis of lipid extract using HCl as a catalyst (On the 3D plot, lanes 1: FAME produced at 5min, 2: FAME produced at 15min, 3: FAME produced at 30min, 4: FAME produced at 60min, 5: FAME produced at 24h, 6: FAME produced at 12h, 7: FAME produced at 72h and 8: FAME standard mix). The lipid extract was obtained from activated sludge (Finham STW, batch 2).

The results show that the amount of acylglycerols (monoacylglycerol, 1, 2-diacylglycerol and 1, 3-diacylglycerol) increased from 5 min and attained a maximum at 1 h before declining to 72 hr, see Figure 3-13, as have been explained above.

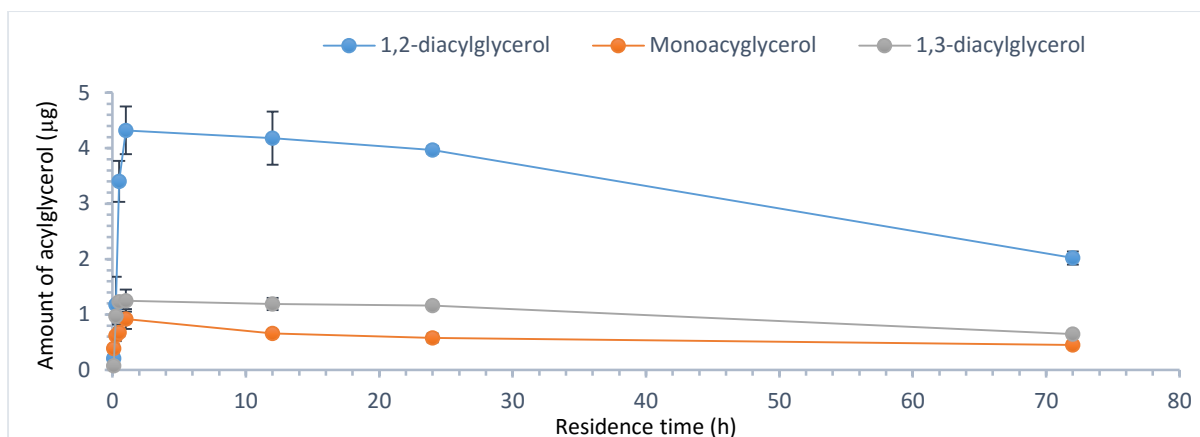


Figure 3-13: illustrating the effect of the residence time on degradation of acylglycerol during the methanolysis of lipid extract from Finham activated sludge (batch 2) catalysed by HCl

2. Investigating the impact of catalyst types on yield of the FAMES produced during the methanolysis of the lipid extract from the activated sludge

The efficacy of catalyst types: boron trichloride (BCl_3) and hydrochloric acid (HCl) on the methanolysis of lipid extract from activated sludge (Finham STW, batch 2) was studied, in order to determine which was capable of producing a high yield of FAMES. Acidic catalysts were considered due to high percentage of free fatty acid 21% obtained from the lipid extract, as using basic catalysts would lead to soap formation, the resultant effects of which are difficulty in the separation and washing of the glycerol (Atabani et al., 2013, Kargbo, 2010, Karmakar et al., 2010). Hydrochloric acid (HCl) out of the three most commonly used acid, is mostly preferred to sulphuric acid (H_2SO_4) and boron trifluoride (BF_3), as it can produce a quantitative FAME yield, it is mild and can easily be separated out of the reaction mix (Lall et al., 2009, Sheppard and Iverson, 1975). In addition, unlike H_2SO_4 , HCl does not decompose polyunsaturated fatty acids (Christie, 1989). HCl, like BF_3 has a low transesterifying than methylating power, this can be increased at a residence time greater than 30min (Carvalho et al., 2004). Boron trichloride (BCl_3), is analogous to BF_3 , but, it is more stable at room temperature, produces less losses of saturated acid and less contaminant (Brian and Gardner, 1967, Christie, 1993, Klopfenstein, 1971).

The experiment was conducted at an optimum residence time of 60 min, based on the result of previous work (see Section 3.2.3.2.6), according to the method presented in Section 2.4. The FAMEs were produced in triplicate ($n = 3$) and the results obtained with their respective standard error of the means (SEM) are presented in Figure 3-14. The results show that although, BCl_3 and HCl catalysed methanolysis of lipid extract, the later catalyst gave a slightly higher yield. Thus, HCl was preferred to BCl_3 .

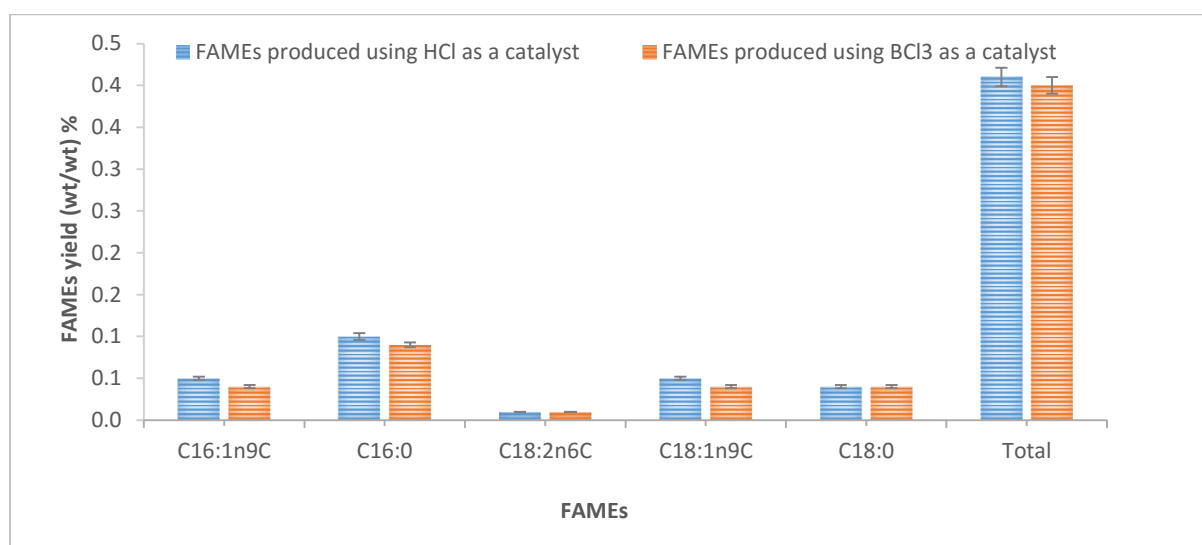


Figure 3-14: effect of catalyst types on the FAMEs yield (wt/wt % dry activated sludge) from the methanolysis reaction of the lipid extract from the activated sludge (palmitic acid (C16:0), palmitoleic acid (C16:1n9C), linoleic acid (C18:2n6C), oleic acid (C18:1n9C) and stearic acid (C18:0)). The lipid extract used was obtained from Finham STW (batch 2).

There was no appreciable difference in the FAMEs yield (dry activated sludge) obtained from HCl -methanolic reagent and freshly opened BCl_3 -methanolic reagent, although, the former reagent gave 0.41 ± 0.011 (wt/wt) % and the later 0.4 ± 0.01 (wt/wt) %, see Figure 3-14. The slight increase in FAMEs yield is attributed to the increased transesterification power of the HCl -methanolic reagent at a residence time greater than 30min, in this instance 60 min (Carvalho et al., 2004).

3.2.3.2.4 Comparing the degree of saturation of fatty acids of the esters produced from the methanolysis of lipid from activated sludge

The percentage composition of saturated and unsaturated fatty acids of the esters produced from methanolysis of lipid extract in Section 3.2.3.2.6 was evaluated with respect to residence times 0.08, 0.5, 1, 12 and 24 h, in order to determine which of them was present in higher proportion. This would provide information regarding the property of the biodiesel. The results obtained are presented in Figure 3-15 and they show that unsaturated fatty acid had a higher proportion in the lipids extract compared to the saturated. These are in agreement with those presented by Pastore et al. (2013) and Revellame et al. (2010). The predominance of the unsaturated fatty acid indicated that the lipid extract and hence the feedstock activated sludge, would be a good substrate for the production of biodiesel, as the tendency to form gel at a very low temperature would be reduced (Soriano et al., 2006). Although, biodiesel with 71.3% oleic acid and 21.4% linoleic acid, according to Bringe (2005) show good qualities such as cold flow, cetane number and oxidative stability, those with very high proportion of palmitoleic acid methyl ester are better and hence best suitable for application in a cold temperature (Knothe, 2005). Thus, the higher percentage of methyl ester of palmitoleic acid compared to that of oleic acid was also an indication that the biodiesel produced from the activated sludge was of high quality.

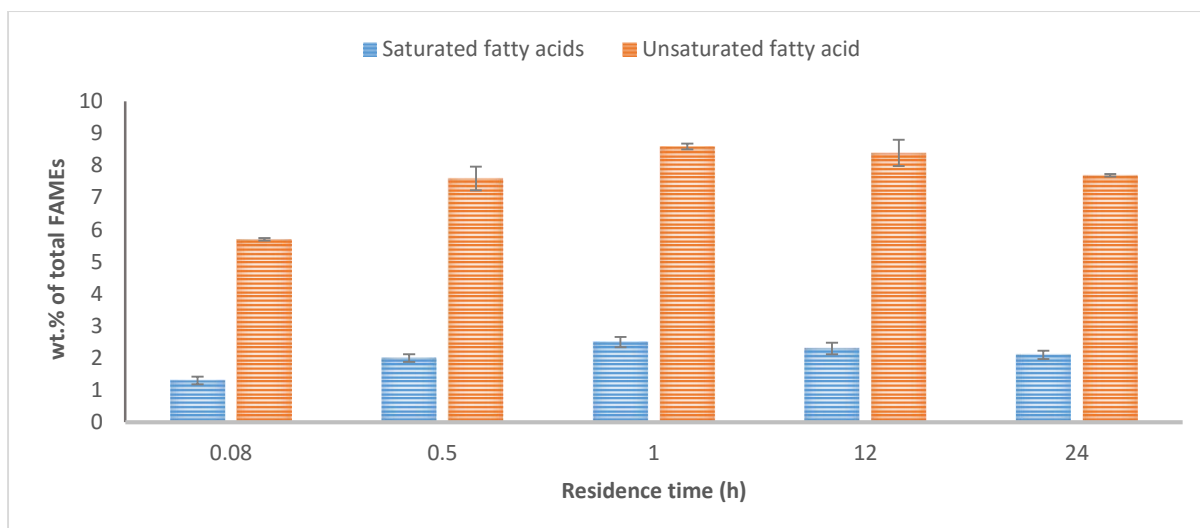


Figure 3-15: comparing the degree of saturation of fatty acids from the lipid extract obtained from activated sludge (Finham STW, batch 2) at different residence times

3.3 CONCLUSIONS

The activated sludge from two sewage treatment works has been characterized and its lipid content evaluated. The solid biomass content varied from suspended solid, total dissolved solid, season and geographical region where the activated sludge slurry was obtained. The utility of Soxhlet method to extract lipid from activated sludge proved to give more yield than Folch et al. (1957) method, although, required large amount of activated sludge, long extraction time and high volume of organic solvent, thus, offering a preference for the later method. More lipid could be extracted from the TSS biomass than TDS. The presence of lipid classes: sterol, wax ester, acylglycerol, free fatty acid and phospholipids, in the lipid extract, presents activated sludge as a potential feedstock for oleochemicals and bioenergy (biodiesel and renewable diesel) production. The maximum percentage of the FAMES from the lipid extract was 13.85 (wt. %) and this corresponded to a yield of 0.41 (wt/wt, on dry activated sludge basis) which was produced at optimum residence time of 1h using 3 mL of ~ 1.25 M HCl at 85 °C. Although, FAMES were produced using activated sludge known to have a low lipid yield and considering

other factors such as method of production, methanol-to-lipid (sludge) ratio, temperature, residence time, water content and % free fatty acid which affect FAMES yield, there is need to develop a new method which has the capacity for maximum extraction. This is important if activated sludge were to be considered as an economical feedstock for bioenergy (biodiesel and renewable diesel) and oleochemical production. To achieve this, subcritical water mediated extraction was investigated and the operating parameters optimised, see Chapter 4.

4 CHAPTER 4 OPTIMISATION OF THE

SUBCRITICAL WATER MEDIATED EXTRACTION

OF LIPID FROM ACTIVATED SLUDGE

4.1 INTRODUCTION

Lipid from activated sludge has been gaining attention in the recent time as a potential feedstock for biodiesel production. Activated sludge is a residue obtained from the biological treatment of wastewater. It contains microorganisms, colloidal matter, organic polymer, carbohydrate, protein and mineral particle which are bonded into a complex matrix (Anderson, 2004, Metcalf and Eddy, 2003). The cell membrane of the microorganisms in activated sludge is known to contain lipids such as phospholipid, steroids and fatty acids (Jarde et al., 2005).

At the moment, most of the few works done on lipid extraction from activated sludge were carried out using organic solvents (Chapter 3). These solvents are not able to recover effectively all the lipids in activated sludge, as they lack the potential to break the cell wall, hydrolyse the cell membrane or weaken the binding forces holding lipids in the matrix of organic and inorganic substances (Huynh et al., 2010, Nguyen et al., 2013). Hence, there is need to develop a benign method which can mimic the properties of these organic solvents and has the capability to recover total lipid from activated sludge. Water at its critical condition 100 – 374°C and pressure sufficient to sustain it in liquid state exhibits these properties (Brunner, 2009, Kruse and Dinjus, 2007). At these conditions, the dielectric constant of water decreases leading to the reduction in polarity and thus, making it possible for lipid extraction. Very few works, have been done on the impact of subcritical water to extract lipid from activated sludge.

Huynh et al. (2010) and Nguyen et al. (2013) have reported that subcritical water can enhance the extractable lipid from activated sludge. Huynh et al. (2010) investigated the impact on neutral lipids after subcritical water pre-treatment on activated sludge. Although, an increase of about four times was obtained, but, they discarded the liquid phase separated from the activated sludge product mix, post subcritical water treatment, probably leading to a loss of a fraction of the neutral lipids

In the current study, we have chosen to use subcritical water to enhance and maximize the lipid yields from activated sludge presented in Chapter 3. As shown in Chapter 3, the maximum lipid yield obtained from activated sludge Finham sewage treatment works (batch 1) was 3.97 (wt/wt) % using methanol (Soxhlet apparatus) and that from Minworth (batch 1) was 7.4 (wt/wt) % using chloroform/methanol (2/1, v/v; Folch et al. (1957)). This was done by investigating the impact of subcritical water mediated extraction of lipid from activated sludge under the influence of operating parameters: temperature, extraction time and biomass loading on lipid yield, composition and biomass solubilisation. We explored the effect of these operating parameters and their interactions using Face Centred Central Composite Design (FCCCD) of Design of Experiment (DoE). FCCCD was used at different levels of temperature (80, 180 and 280°C), extraction time (20, 50 and 80 min) and biomass loading (1, 5 and 8%) to generate different experimental conditions. The lipid distributions in both liquid and solid products post subcritical water treatment was obtained. Total lipid yield and compositions were obtained by combining the lipids from these products. The Percentage of activated sludge biomass solubilised was also obtained. The results (responses) were analysed using the statistical analysis inbuilt function in Design Expert Software 7.0 2005. The model equations and graphs of the responses were obtained and the former was used to explore the design spaces, in order to predict lipid yield, composition and biomass solubilisation. The models were validated through the predicted responses using the experimental data.

4.2 RESULTS AND DISCUSSIONS

4.2.1 Evaluation of solvents for the recovery of palmitic acid and vegetable oil from water

In order to evaluate the efficiency of the method which was developed for lipid recovery post subcritical water mediated extraction from activated sludge, a model experiment was carried out using palmitic acid and vegetable oil, as shown in Chapter 2.5. The efficacy of solvents: hexane, diethyl ether and toluene in recovering palmitic acid and vegetable oil from water was investigated. 1 mL of distilled water was added to 5mg of palmitic acid and vegetable oil, respectively and vortexed. The palmitic acid and vegetable oil were recovered according to the method described in Sections 2.5.2.1 and 2.5.2.2, respectively. The results revealed that hexane was a better solvent for recovering palmitic acid and vegetable oil from water compared to diethyl ether and toluene (Figure 4-1 and Section 4.2.7.1). The degree of non-polarity and

Table 4-1: free fatty acid and vegetable oil recovery using different organic solvents

S/N	Solvent	Recovery (wt/wt) %	
		Vegetable oil	Palmitic acid
1	Hexane	100.0 ± 0.0	99.0 ± 0.7
2	Diethyl ether	87.0 ± 1.3	*119.0 ± 0.1
3	Toluene	97.3 ± 1.3	97.3 ± 3.7

(mean ± SEM; n = 3; S/N = serial number and * is greater than the expected 100 (wt/wt) % as water seemed to have been bound to the interstices of palmitic acid and it was very difficult to evaporate)

volatility (hexane > toluene > diethyl ether) affects lipid extraction (Boocock et al., 1992, Byers, 2003, Liu, 1994, Reichardt, 2003). A highly non polar organic solvents used to dissolve lipid, evaporates faster due to high volatility compared to a less non-polar solvent. Vegetable oil contains predominantly triglyceride which is known to be a non-polar substance just like palmitic acid and would dissolve effectively in a non-polar solvent. Thus, hexane, followed by toluene then diethyl ether will extract more vegetable oil and palmitic acid and can be evaporated quickly at moderate temperatures. Although, diethyl ether recovered 119 ± 0.09 (wt/wt) % which is greater than the expected 100 % probably due to water contained in the interstices of palmitic acid, it was very difficult to evaporate. Hence, hexane is more suitable since it recovered effectively vegetable oil and palmitic acid, and was efficiently evaporated.

4.2.2 Preliminary experiment on the subcritical water mediated extraction of lipid from the activated sludge

This was conducted to determine the potential of extracting lipids from the products of subcritical water mediated extraction. At the end of the subcritical water treatment, liquid and solid products were obtained by filtration, see Sections 4.2.2.1 and 4.2.2.2.

4.2.2.1 Investigating the potential of extracting lipid from the liquid product post subcritical water mediated extraction

Experiments were carried out to determine the efficacy of extracting lipid from the liquid product post subcritical water treatment on activated sludge (Finham STW, batch 2), according to the methods presented in Sections 2.6.1 and 2.5.2. The lipid yields obtained gravimetrically were 0.74 ± 0.02 (wt/wt) % at 80 °C and 0.67 ± 0.1 (wt/wt) % at 140 °C, respectively. The results show that the difference in the lipid yield obtained at 80°C and 140°C, under a constant extraction time 47min and activated sludge biomass loading 0.7g was 0.07 (wt/wt) %. Thus,

subcritical water treatment on activated sludge at these temperatures might not have an appreciable impact on lipid yield.

4.2.2.2 Investigating the potential of extracting lipid from the solid product post subcritical water mediated extraction

The efficacy of obtaining lipids from the solid product post subcritical water treatment on activated sludge (Minworth STW, batch 1) was investigated. The subcritical water mediated extraction was carried out at temperature 280°C, residence time 40 min, 5% biomass and 15mL water loadings, according to the methods prescribed in Section 2.6.1 and lipid was extracted from the solid product by Folch et al. (1957) method, see Section 2.3.1.2. The lipid yield obtained from 0.75g activated sludge solid biomass was 13.00 ± 0.9 (wt/wt) % at 280°C (n = 3). This result shows that lipid must be extracted from both solid products post subcritical water treatment in order to obtain a total yield.

4.2.2.3 Determining the total lipid yield from activated sludge using subcritical water mediated extraction

The total lipid yield from activated sludge (Minworth STW, batch 1) using subcritical water mediated extraction was determined in order to ascertain the lipid content of the biomass. Lipid was extracted from the liquid product using hexane as described in Section 2.5.2 and from the solid by Folch method, see Section 2.3.1.2. It was expected that lipids from the liquid product would be mostly neutral lipids and those from the solid product would be a combination of neutral lipids and phospholipids, due to the polarities of the solvents used (Section 3.3.2). The highest lipid yield from liquid products was 5.2 ± 0.23 (wt/wt) % and that from solid 13.00 ± 0.9 (wt/wt) % obtained at 280°C (Table 4-2). The results showed that the lipid content in the solid product was higher than that from liquid, post subcritical water extraction. The total lipid yield shows a dependency on temperature. Thus, 18.2 ± 1.13 (wt/wt) % of lipid was obtained

at 280°C compared to $8.5 \pm 0.12\%$ at 80°C. This result was compared to the lipid yield 3.92 ± 2.34 (wt/wt) % obtained using methanol (Soxhlet apparatus). The comparison shows that the

Table 4-2: total lipid yield post subcritical water mediated extraction from activated sludge obtained from Minworth STW (batch 1)

S/N	Temperature (°C)	Lipid yield (wt/wt) %		
		Liquid product	Solid product	Total lipid
1	80	1.5 ± 0.12	7.0 ± 0.0	8.5 ± 0.12
2	140	1.6 ± 0.06	8.00 ± 0.3	9.6 ± 0.36
3	280	5.2 ± 0.23	13.00 ± 0.9	18.2 ± 1.13

(mean \pm SEM; $n = 3$ and S/N = serial number).

lipid yield increased by 4.6 times with the subcritical water treatment. The increased yield could be attributed to high mass transfer between lipid and water caused by the hydrolysis of cellulose or hemi-cellulose of the activated sludge biomass as the extraction was been carried out (Go et al., 2014). The increased mass transfer helped in increasing the rate of the lipid released by breaking down of the bond binding it to protein, carbohydrate and mineral (Anderson, 2004). The yield is also suspected to have been increased due to the ability of water under subcritical condition to function as catalyst in hydrolysing the activated sludge biomass (Galkin and Lunin, 2005, Quitain et al., 2002).

The increase in lipid yield using subcritical water treatment obtained in this study was higher than those recorded by Nguyen et al. (2013) and Huynh et al. (2010). This higher lipid yield might be due to difference in activated sludge source and a lower temperature 175°C and residence time 15 min used by Nguyen et al. (2013) and Huynh et al. (2010) which gave them an increase 1.8 - 3.7 times, post subcritical water treatment. As will be seen later in this report,

lipid yield of activated sludge under the subcritical conditions depends on the operating parameters such as temperature, extraction time and biomass loading.

Generally, it has been demonstrated in this study that there is a need for subcritical water treatment if lipid from activated sludge is to be considered in the bioenergy (biodiesel and renewable diesel) and oleochemicals production, as solvent extraction alone does not give a full recovery.

4.2.3 Results of one-factor-at-a-time (OFAT) experiment

OFAT experiment was carried out as a scoping experiment to determine the impact of operating parameters post subcritical water mediated extraction on activated sludge (Finham STW, batch 2) and hence obtain their boundary limits (upper and lower) in relation to the lipid yield, which will form the basis for the Design of Experiment (DoE). The operating parameters were biomass loading (1, 5 and 8%), extraction time (20, 40 and 80 min) and temperature (80, 140 and 280°C) and experiments were conducted, according to the method presented in Section 2.6.2. The results were obtained in terms of the lipid yields and the operating parameters: biomass loading, residence time and temperature, see Figures 4-1, 4-2 and 4-3. It was observed that under constant water volume of 15 mL, temperature 140°C and residence time 40 min, 1% activated sludge biomass loading favoured a higher lipid yield of $9.6 \pm 0.3\%$ (wt/wt) % compared to 5 and 8%, respectively (Figure 4-1). Thus, lower biomass loading was required in order to extract high yield of lipid from activated sludge in a given volume of water. This result is in agreement with that obtained by Go et al. (2014) and Reddy et al. (2014). At a lower biomass loading 1% or higher water content, it was found that a maximum lipid yield 9.6 ± 0.3 (wt/wt) % was obtained, under constant temperature 140°C, water volume 15 mL and extraction time 40 min, see Figure 4-1. The increase in the extractable lipid was probably due to quick disruption of the cell walls of the activated sludge biomass, thereby increasing the

surface area of the lipids exposed for extraction and hence, high yield will be obtained (Nguyen et al., 2013).

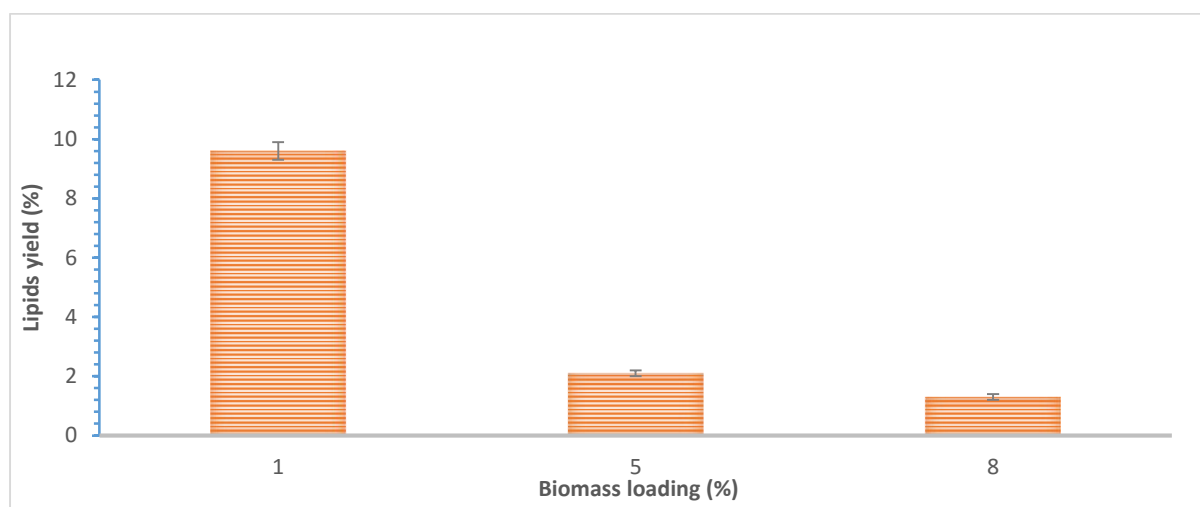


Figure 4-1: effect of biomass loading on the subcritical water mediated lipid extraction from activated sludge (Finham STW, batch 2) at constant temperature 140°C and extraction time 40 min

Extraction time was also found to have impacted the lipid yield as shown in Figure 4-2. It was established that increase in the extraction time 20, 40 and 80 min resulted to a corresponding increase in the lipid yield 0.6 ± 0.1 (wt/wt) %, 1.0 ± 0.12 (wt/wt) % and 1.4 ± 0.1 (wt/wt) % at a constant temperature 140°C and biomass loading 5%. The increased yield was probably due to longer residence time required for disrupting the cell wall before the lipid was exposed for easy extraction (Tsigie et al., 2012b). This observation was corroborated with the results shown by Z. Liu et al. (2014) in which it was presented that there was an increase in oil yield with respect to extraction time 30, 40, 50 and 60 min.

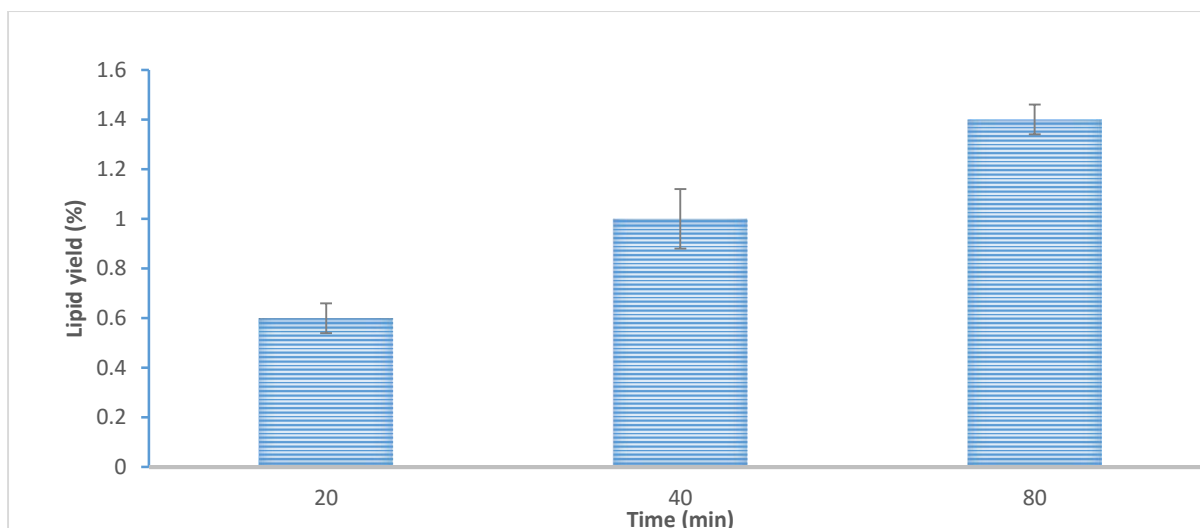


Figure 4-2: illustration of the effect of time on the subcritical water mediated lipid extraction from the activated sludge (Finham STW, batch 2) at constant temperature 140°C and biomass loading 5%

It was demonstrated in Figure 4-3 that increase in temperature 80, 140 and 280°C resulted to a corresponding increase in lipid yield 1.5 ± 0.12 (wt/wt) %, 1.6 ± 0.06 (wt/wt) % and 5.2 ± 0.23 (wt/wt) % under constant biomass loading 5%, water volume 15 mL and extraction time 40 min. At higher temperature, the hydrogen bond in water dissociates and there is also increased level of interaction with lipids, thereby increasing its solubility and hence, favouring the extraction of non-polar lipids substances (Brunner, 2009, Peterson et al., 2008). Thus, the increased lipid yield at 280°C resulted from the extraction of more non-polar lipids at this temperature.

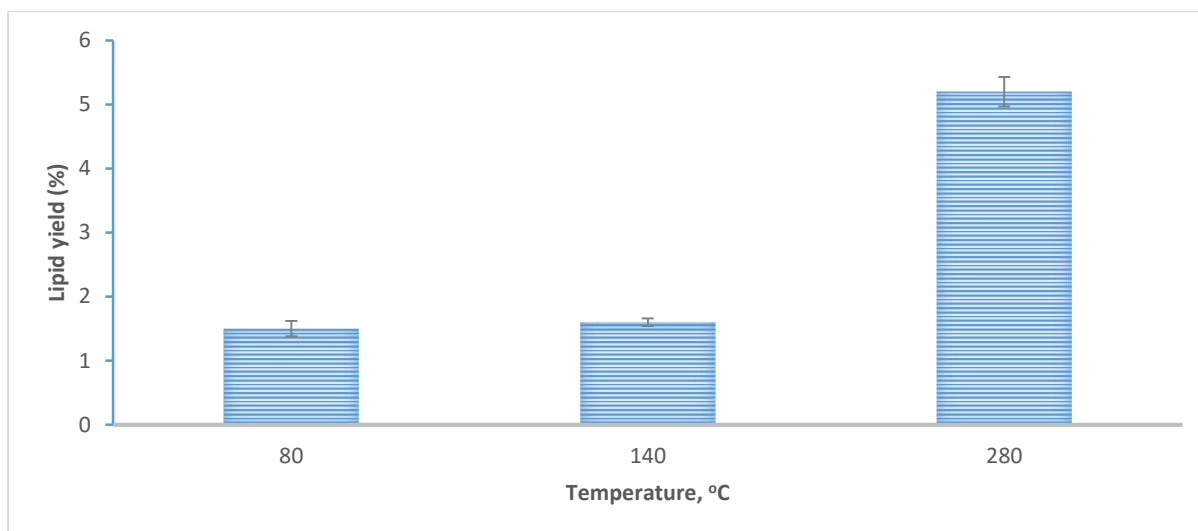


Figure 4-3: effect of temperature on the subcritical water mediated extraction of lipid from activated sludge (Finham STW, batch 2) at constant extraction time 40 min and biomass loading 5%

4.2.4 Design of Experiment (DOE)

DOE was used to evaluate the impact of the operating parameters: temperature, extraction time and biomass loading which was suggested by the OFAT experiment as influencing the lipid yield from activated sludge (Minworth STW, batch 1). The effects of these parameters on lipid composition and biomass solubilisation were also investigated. Based on the OFAT results (4.2.3), the following parameter boundary limits: temperature (80 – 280°C), extraction time (20 – 80 min) and biomass loading (1 – 8%), were used in the experimental design, see Section 2.6.3. The type of DOE used was Face Centred Central Composite Design (FCCCD) and the experiment was designed in the Design Expert 7.0 2005 Software environment.

4.2.4.1 Face centred central composite design (FCCCD)

As mentioned in Section 4.2.2.3, the products of subcritical water mediated extraction was separated into liquid and solid phases. Lipid was extracted from the liquid phase using hexane, with this solvent, mostly neutral lipids were expected (Section 2.5.2) and from the solid phase

using chloroform/methanol (2/1, v/v) (Folch method) which is known to extract a combination of neutral lipid and phospholipids (Smedes and Askland, 1999), see Sections 2.3.1.2. The lipids obtained from each phase depended on the polarity of the organic solvent, see Section 3.3.2 for further discussion. The results of the lipid yields from both phases at different operating conditions were combined and analysed using Response Surface Methodology (RSM). The FCCCD factors levels and lipid yield from both liquid and solid products are presented in Table 4-3. Comparatively, more lipid was extracted from the solid product than from liquid, post subcritical water extraction, See Table 4-3. This thus, validates the earlier results presented in Section 4.2.3. The percent of biomass solubilised at different operating condition were obtained by gravimetry, after drying to a constant weight. The results of the total lipid yield and biomass solubilised obtained are presented in Table 4-4. These show that a maximum lipid yield of 41 (wt/wt) % was obtained at the optimum operating conditions of temperature 80°C, extraction time 20 min and biomass loading 1%. The maximum biomass solubilised of 58.1 (wt/wt) % was obtained at temperature 280°C, time 80 min and biomass loading 8%.

Table 4-3: lipid yield recovered from the products of the subcritical water mediated extraction using FCCCD (the activated sludge used was obtained from Minworth STW, batch 1)

Run	Tempera- ture (°C)	Time (min)	Weight of biomass (mg) or biomass loading (%)	Lipid weights (mg)			% lipid yield	
				liquid product	solid product	total	liquid product	Solid product
1	180	50	680 (4.5)	21	45.8	66.8	3.1	6.7
2	280	20	1200 (8.0)	15.1	75.1	90.2	1.3	6.3
3	180	50	680 (4.5)	30.5	83	113.5	4.5	12.2
4	280	80	150 (1.0)	15.2	24.3	39.5	10.1	16.2
5	280	20	150 (1.0)	23.8	29.9	53.7	15.9	19.9
6	80	20	1200 (8.0)	12.6	78.6	91.2	1.1	6.6
7	180	50	680 (4.5)	32.4	69.3	101.7	4.8	10.2
8	80	80	1200 (8.0)	10.9	73.8	84.7	0.9	6.2
9	80	20	150 (1.0)	28.3	33.2	61.5	18.9	22.1
10	280	80	1200 (8.0)	32.9	187.2	220.1	2.7	15.6
11	80	80	150 (1.0)	25.1	20.3	45.4	16.7	13.5
12	180	50	680 (4.5)	23.4	65.6	89	3.4	9.6
13	180	50	680 (4.5)	25.5	x	25.5	3.8	X
14	80	50	680 (4.5)	28.6	43.3	71.9	4.2	6.4
15	180	50	150 (1.0)	26.8	22.4	49.2	17.9	14.9
16	280	50	680 (4.5)	26.7	112.4	139.1	3.9	16.5
17	180	50	680 (4.5)	16.7	65.5	82.2	2.5	9.6
18	180	20	680 (4.5)	26.6	66.7	93.3	3.9	9.8
19	180	80	680 (4.5)	10.3	71.3	81.6	1.5	10.5
20	180	50	1200 (8.0)	12	108.3	120.3	1.0	9.0

(x = the value of the lipid yield from the dried residual activated sludge biomass not available and was not used in the analysis)

Table 4-4: Responses from subcritical water mediated lipid extraction using FCCCD (the activated sludge used was obtained from Minworth STW, batch 1)

Run	Block	Factors			Responses (%)	
		A: Temperature (°C)	B: Time (min)	C: Biomass Loading (%)	Total lipid Yield	Biomass solubilised
1	Day 1	280	20	1.0	35.8	50.0
2	Day 1	180	50	4.5	16.7	47.4
3	Day 1	280	80	8.0	18.3	58.1
4	Day 1	180	50	4.5	15.0	42.6
5	Day 1	80	80	1.0	30.3	40.5
6	Day 1	280	80	1.0	26.3	56.0
7	Day 1	180	50	4.5	9.8	50.6
8	Day 1	80	20	1.0	41.0	26.5
9	Day 1	80	20	8.0	7.6	19.7
10	Day 1	180	50	4.5	13.1	50.9
11	Day 1	280	20	8.0	7.5	54.0
12	Day 1	80	80	8.0	7.1	20.0
13	Day 2	180	80	4.5	12.0	51.1
14	Day 2	180	50	8.0	10.0	48.3
15	Day 2	180	50	4.5	12.1	49.3
16	Day 2	180	50	1.0	32.8	50.7
17	Day 2	80	50	4.5	10.6	19.5
18	Day 2	280	50	4.5	20.5	57.8
19	Day 2	180	20	4.5	13.7	43.3
20	Day 2	180	50	4.5	X	47.8

(x = the value of the lipid yield from the dried residual activated sludge biomass not available and was not used in the analysis)

4.2.4.1.1 Statistical analysis for lipid yield, composition and biomass solubilised responses

This analysis was important in order to produce models which describe the relationship that exists between responses and operating parameters (factors). The fitness of the models to the

experimental data was evaluated using analysis of variance (ANOVA). The statistical analysis of lipid yield, biomass solubilised and lipid composition are presented below

4.2.4.1.1.1 Total lipid yield response

The total lipid yield response was modelled using Design Expert Software 7.0 in terms of linear, two factor interaction (2FI), quadratic and cubic polynomial to obtain the best fittings for the experimental data. The Software compared different R-Squared parameters of the models, standard deviation (Std. Dev.) and PRESS (predicted residual sum of squares). The quadratic model was suggested by the software as the best fitting for the experimental data considered, probably because it showed high R-Squared 0.9591, low Std. Dev. 3.11 and low PRESS 703.36 compared to other models, see Table 4-5. The quadratic model showed the main and interaction effects on the actual factors temperature (A), time (B) and biomass loading (C), see Equation 4-1.

$$\text{Total lipid yields} = 55.55261 - 0.020825 * A - 0.20206 * B - 12.24821 * C + 7.25000E - 003 * A * C + 0.036310 * B * C + 0.64689 * C^2 \quad \text{Equation 4-1}$$

Table 4-5: model summary statistics for total lipid yield response

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	6.15	0.7203	0.6604	0.4329	1075.38	
2FI	5.58	0.8191	0.7204	0.0961	1714.10	
<u>Quadratic</u>	<u>3.11</u>	<u>0.9591</u>	<u>0.9131</u>	<u>0.6291</u>	<u>703.36</u>	<u>Suggested</u>
Cubic	2.69	0.9847	0.9351	-7.3061	15751.53	Aliased

The result of the analysis of variance (ANOVA) showed that p-value was <0.0001 and lack of Fit 0.5109, indicating that the model was valid and gave a good fitting to the experimental data,

see Table 4-6. The biomass loading, temperature-biomass loading, extraction time-biomass loading and squared of biomass loading were significant model terms.

Table 4-6: analysis of variance (ANOVA) for lipid yield response quadratic model

Source	Sum of squares	Df	Mean square	F Value	p-value Prob > F	Significant
Block	42.06	1	42.06			
Model	1790.99	6	298.50	31.15	<0.0001	Significant
A-Temperature	13.92	1	13.92	1.45	0.2533	
B-Time	13.46	1	13.46	1.40	0.2610	
C-Biomass loading	1338.65	1	1338.65	139.71	<0.0001	
AC	51.51	1	51.51	5.38	0.0407	
BC	116.28	1	116.28	12.14	0.0051	
C ²	257.17	1	257.17	26.84	0.0003	
Residual	105.40	11	9.58			
Lack of Fit	79.15	8	9.89	1.13	0.5109	not significant
Pure Error	26.25	3	8.75			
Cor Total	1938.45	18				

The diagnostics analysis was carried out using Design Expert software to validate the quadratic model developed and to identify certain outliers which may have adverse effect on the model. The analysis was done by comparing the actual values of the total lipid yields obtained from the experiment with those predicted by the model. The maximum actual and predicted values of total lipid yield were 41.0 (wt/wt) % and 39.58 (wt/wt) %, respectively, obtained at the optimum conditions of temperature 80°C, extraction time 20 min and biomass loading 1%, See Table 4-7. The residual difference of 1.42 (wt/wt) % shows that actual and predicted values of

the total lipid yield are in close agreement and that the quadratic model fitted the experimental data well.

Table 4-7: Diagnostics Case Statistics for determining the fitness of the model to the experimental data using the percentage residual between the actual and predicted total lipid yield response

Run order	Temp- rature (°C)	Time (min)	Biomass Weight loading (mg) or (%)	Standard order or	Actual value (%)	Predicted value (%)	Residual (%)
8	80	20	150 (1)	1	41.00	39.58	1.42
1	280	20	150 (1)	2	35.80	36.87	-1.07
5	80	80	150 (1)	3	30.30	29.64	0.66
6	280	80	150 (1)	4	26.30	26.92	-0.62
9	80	20	1200 (8)	5	7.60	3.74	3.86
11	280	20	1200 (8)	6	7.50	11.18	-3.68
12	80	80	1200 (8)	7	7.10	9.05	-1.95
3	280	80	1200 (8)	8	18.30	16.48	1.82
7	180	50	680 (4.5)	9	9.80	13.76	-3.96
10	180	50	680 (4.5)	10	13.10	13.76	-0.66
4	180	50	680 (4.5)	11	15.00	13.76	1.24
2	180	50	680 (4.5)	12	16.70	13.76	2.94
17	80	50	680 (4.5)	13	10.60	12.51	-1.91
18	280	50	680 (4.5)	14	20.50	14.87	5.63
19	180	20	680 (4.5)	15	13.70	14.85	-1.15
13	180	80	680 (4.5)	16	12.00	12.53	-0.53
16	180	50	150 (1)	17	32.80	33.19	-0.39
14	180	50	1200 (8)	18	10.00	10.05	-0.047
15	180	20	680 (4.5)	20	12.10	13.69	-1.59

(A). Investigating the effect of interaction of factors on lipid yield

The influence of interaction of factors: temperature, extraction time and biomass loading was studied using Equation 4-1, so as to understand if they had any effects on the lipid yield. The effects of temperature - biomass loading and extraction time – biomass loading were found to be significant. These studies are presented below

(i). Interaction effect of temperature and biomass loading on lipid yield

Temperature was varied at 80, 180 and 280 °C, biomass loading 1, 4.5 and 8 % and the extraction time was kept constant for 50 min using the model Equation 4-1. The model graph obtained from FCCCD response surface is shown in Figure 4-4. The interaction of biomass loading 1% and temperature for extraction time of 50 min produced a lipid yield of 34.58 (wt/wt) %. The result thus, obtained showed that interaction of biomass loading and temperature had a great effect on lipid yield.

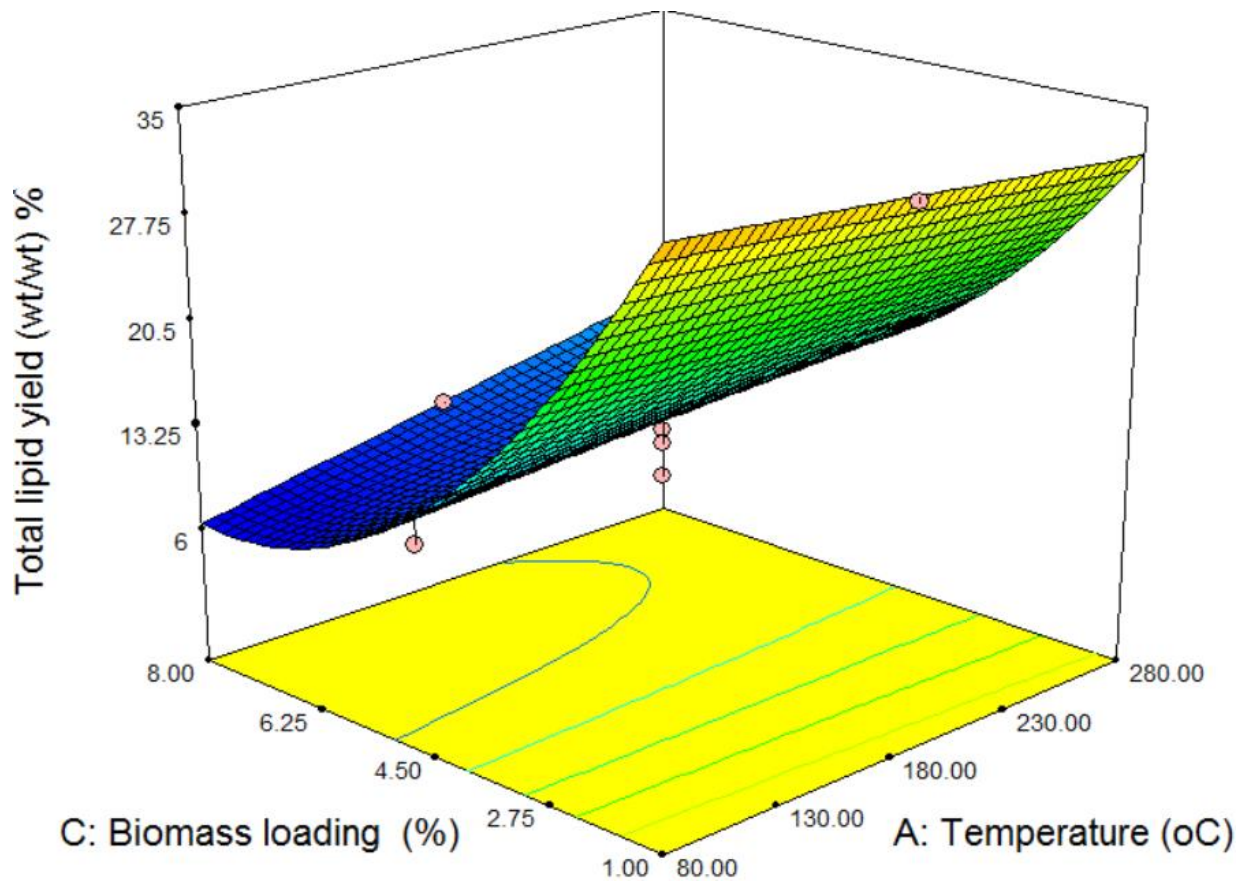


Figure 4-4: illustrating the effect of temperature and biomass loading interaction on the lipid yield at constant extraction time 50 min

(ii). Interaction effect of time and biomass loading on lipid yield

FCCCD response surface model and graph of interaction effect of time and biomass loading obtained are presented in Equation 4-1 and Figure 4-5, respectively. The model graph was obtained at varied extraction time 20, 50 and 80 min and biomass loading 1, 4.5 and 8% and the temperature was kept constant at 180°C. The interaction of biomass loading 1% and time 20 min at temperature of 180 °C resulted to a lipid yield response of 38.19 (wt/wt) %. The result showed that the interaction of the biomass loading and residence time influenced the lipid yield.

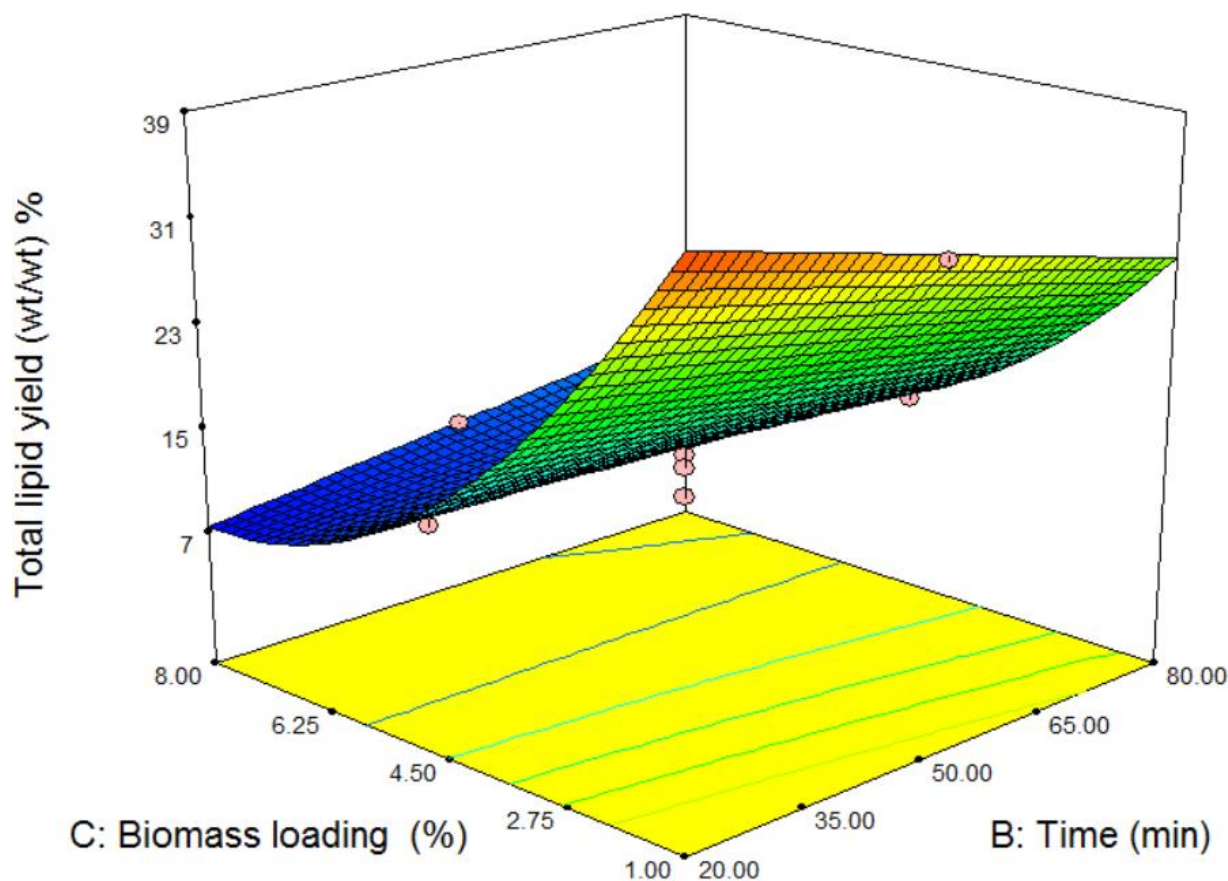


Figure 4-5: illustrating the effect of time and biomass loading interaction on the lipid yield at constant temperature 180°C

Generally, the optimum lipid yield 41.0 (wt/wt) % was obtained at temperature 80°C, extraction time 20 min and biomass loading 1%, resulting to 454.1(wt/wt) % increase to that observed without subcritical water treatment and optimization 7.4 (wt/wt) %. The advantage of which is increase in the lipid available for bioenergy (biodiesel and renewable diesel) and oleochemical production at a cheaper cost, since the extraction is carried out at minimum operating conditions of temperature 80°C, extraction time 20 min and biomass loading 1%.

(B). Validating the quadratic model through experiment using optimum conditions generated by Design-Expert Software

The lowest and highest total lipid yields obtained from FCCCD response surface were 7.1 and 41.0 (wt/wt) %. This total lipid yield was maximised using numerical optimization in the Design-Expert Software 7.0 2005. Twenty solutions of the optimal conditions were generated with desirability between 0.937 and 0.487. The optimum conditions selected were temperature 280 °C, extraction time 29.2 min and biomass loading 1 %. The total lipid yield predicted by the model was 36.84 (wt/wt) % and that obtained from experiment under the optimum conditions was 28.7 (wt/wt) %. This disparity was expected since the sources of the activated sludge were different. The actual value of the lipid yield was obtained using activated sludge from Finham sewage treatment works which was known to contain a lower lipid yield 3.97% compared to that from Minworth 7.4 % used in the design of experiment, see Section 3.2.2.

4.2.4.1.1.2 Biomass solubilisation response

The activated sludge biomass solubilised under the experimental conditions considered, see Table 4-8 was modelled to determine the impact of operating parameters on solubilisation and whether it had a relationship with lipid yield. The modelling was carried out using FCCCD in Design-Expert Software 7.0.0 2005. The model summary statistics obtained from the FCCCD response surface is presented in Table 4-8. R-Squared parameters, Std. Dev. and PRESS obtained from linear, 2FI, quadratic and cubic models were compared automatically by the Software. The Software suggested a quadratic model because, it had high R-Squared 0.9621, low Std. Dev. 3.54 and low PRESS 942.53, see Table 4-8. The quadratic model obtained is presented in terms of temperature (A), extraction time (B) and biomass loading (C), see Equation 4-2. The model showed that temperature had a greater impact in the biomass solubilisation compared to extraction time and biomass loading. This is not a surprise as

$$\text{Biomass solubilised (\%)} = -2.02659 + 0.39021 * A + 0.19090 * B - 1.89286 * C + 0.011929 * A * C - 0.018571 * B * C - 8.17200E - 004 * A^2 \quad \text{Equation 4-2}$$

solubility of water increases with temperature giving rise to high biomass solubilisation. At 80°C, the biomass solubilised was 19.5 (wt/wt) %, this increased through 180°C to 280°C giving rise to 57.8 (wt/wt) %. The low amount of the biomass solubilised suggested that 80°C was too weak to break the cell wall of the activated sludge (biomass), unlike the high value that was obtained at 280°C. This result is in agreement with that obtained by Passos et al. (2013) in which they stated that solubilisation increases with temperature. Biomass solubilisation increased from 43.3 (wt/wt) % to 51.1 (wt/wt) % with extraction time 20, 50, 80 min at constant temperature 180°C and biomass loading 4.5%. This increase was steep from 20 to 50 min, after which it remained constant before increasing slightly at 80 min. It is expected that a long extraction time may have resulted to increased dissolution of activated sludge particles under subcritical water, the effect of which was rise in biomass solubilisation. This observation was supported by Passos et al. (2013) as they reported that a longer pre-treatment time was necessary in order to obtain high biomass solubilisation. The main effect of biomass loading 1, 4.5, 8% when investigated using the quadratic model at constant temperature 180°C and extraction time 50 min resulted to a decrease in biomass solubilisation from 50.6 (wt/wt) % to 42.6 (wt/wt) %. Under these operating conditions, 1% biomass loading was sufficient in order to obtain high biomass solubilisation probably due to increased surface area.

Table 4-8: model summary statistics for biomass solubilised response

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	6.18	0.8073	0.7688	0.6232	1120.43	
2FI	5.78	0.8652	0.7977	0.4142	1741.72	
<u>Quadratic</u>	<u>3.54</u>	<u>0.9621</u>	<u>0.9241</u>	<u>0.6830</u>	<u>942.53</u>	<u>Suggested</u>
Cubic	3.07	0.9841	0.9429	-0.6766	4985.08	Aliased

Analysis of variance (ANOVA) was used to evaluate the fitness of the model to experimental data, see Table 4-9. The p-values and Lack-of-Fit of <0.0001 and 0.6027 respectively, revealed that the model was significant and Lack-of-Fit was insignificant. The model thus, was the best fitting for the experimental data.

Table 4-9: Analysis of variance (ANOVA) for biomass solubilised response quadratic model

Source	Sum of squares	Df	Mean square	F Value	p-value Prob > F	Significant
Block	41.77	1	41.77			
Model	2848.51	6	474.75	45.62	<0.0001	Significant
A-Temperature	2241.01	1	2241.01	215.37	<0.0001	
B-Time	103.68	1	103.68	9.96	0.0083	
C-Biomass loading	55.70	1	55.70	5.35	<0.0392	
AC	139.45	1	139.45	13.40	0.0033	
BC	30.42	1	30.42	2.92	0.1130	
A ²	278.26	1	278.26	26.74	0.0002	
Residual	124.87	12	10.41			
Lack of Fit	79.11	8	9.89	0.86	0.6027	not significant
Pure Error	45.75	4	11.44			
Cor Total	3015.15	19				

In order to evaluate the presence of outliers which could impact negatively on the model, diagnostic case statistics was carried out using Design-Expert Software 7.0.0. The difference between the predicted and actual lipid yields obtained by the software and experimentally, respectively, under the same conditions of temperature, extraction time and biomass loading gave the percent residual. The maximum actual and predicted values of the biomass solubilised were 58.10 and 58.36 (wt/wt) %, respectively, obtained at the optimum conditions temperature 280 °C, extraction time 80 min and biomass loading 8 %, see Table 4-10. Thus, residual 0.26 (wt/wt) (%) is also a good indication that the model fitted the experimental data well.

Table 4-10: diagnostics case statistics for biomass solubilised response to identify outliers and improve the model obtained

Run order	Temp- rature (°C)	Time (min)	Biomass Weight or loading (mg or %)	Standard order	Actual value (%)	Predicted value (wt/wt)%	Residual (wt/wt) %
7	80	20	150 (1)	1	26.50	26.70	-0.20
8	280	20	150 (1)	2	50.00	48.29	1.71
9	80	80	150 (1)	3	40.50	37.04	3.46
10	280	80	150 (1)	4	56.00	58.63	-2.63
11	80	20	1200 (8)	5	19.70	17.53	2.17
12	280	20	1200 (8)	6	54.00	55.82	-1.82
13	80	80	1200 (8)	7	20.00	20.07	-0.066
14	280	80	1200 (8)	8	58.10	58.36	-0.26
1	180	50	680 (4.5)	9	50.60	48.47	2.13
2	180	50	680 (4.5)	10	50.90	48.47	2.43
3	180	50	680 (4.5)	11	42.60	48.47	-5.87
4	180	50	680 (4.5)	12	47.40	48.47	-1.07
15	80	50	680 (4.5)	13	19.50	24.88	-5.38
16	280	50	680 (4.5)	14	57.80	54.82	2.98
17	180	20	680 (4.5)	15	43.30	44.80	-1.50
18	180	80	680 (4.5)	16	51.10	51.24	-0.14
19	180	50	150 (1)	17	50.70	50.38	0.32
20	180	50	1200 (8)	18	48.30	45.66	2.64
5	180	50	680 (4.5)	19	47.80	48.02	-0.22
6	280	20	1200 (8)	20	49.30	48.02	1.28

(A). Investigating the effect of factors interaction on biomass solubilisation

This was necessary to develop an understanding on whether the interaction of temperature, extraction time and biomass loading had an impact on biomass solubilisation. The model:

Equation 4-2 predicted that temperature – biomass loading and extraction time – biomass loading significantly increased the biomass solubilisation (response).

(i). Interaction effect of temperature and biomass loading

The interaction effect of temperature and biomass loading was studied based on the quadratic model obtained from the FCCCD using Design-Expert 7.0.0, 2005 in order to determine if it had any influence on the response solubilisation. The temperatures were 80, 180 and 280°C and biomass loadings 1, 4.5 and 8% while the actual factor extraction time was 50 min. The interaction effect of temperature and biomass loading gave a maximum value of biomass solubilised 57.8 (wt/wt) % and minimum 19.5 (wt/wt) %. The maximum value of biomass solubilized were obtained at temperature 280 °C, biomass loading 4.5 %, and extraction time 50 min. The minimum value of biomass solubilised were obtained at temperature 80 °C, biomass loading 4.5 % and extraction time 50 min. The model graph obtained is presented in Figure 4-6 and it showed that temperature – biomass loading interaction influenced the degree of response solubilisation.

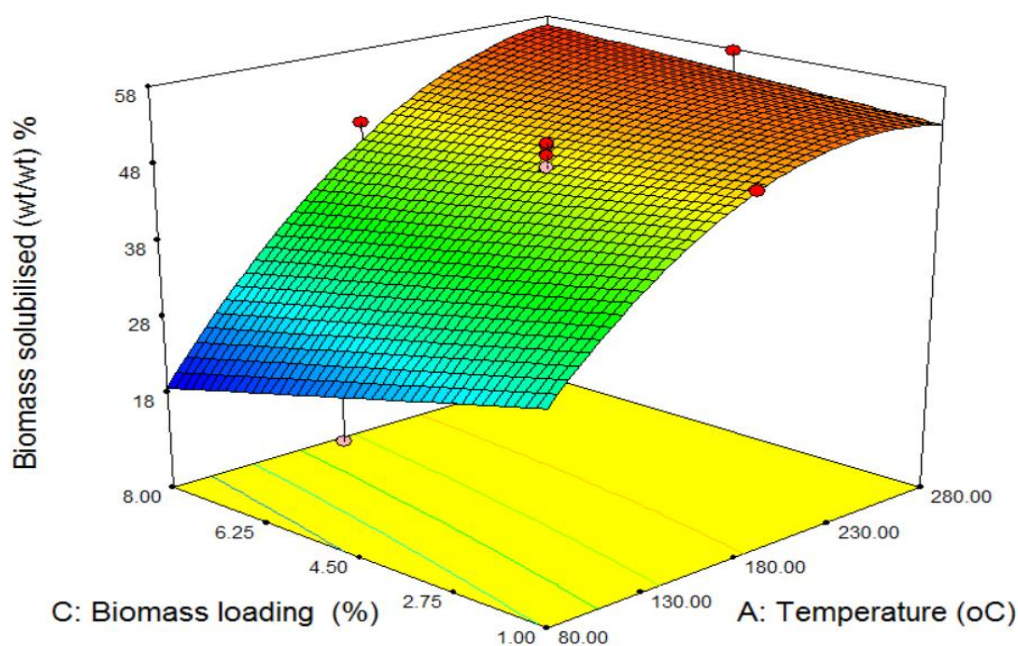


Figure 4-6: a 3D plot of the interaction effect of temperature and biomass loading on solubilisation at constant extraction 50 min

(ii). Interaction effect of time and biomass loading

The impact of the interaction between time and biomass loading was investigated using the experimental data obtained in the Design-Expert software in order to determine if there was any influence on the response factor (biomass solubilisation). The 3D model graph obtained at varied extraction time 20, 50 and 80 min, biomass loading 1, 4.5 and 8% and constant temperature 280°C is shown in Figure 4-7. The maximum and minimum values of biomass solubilized were 50.7 (wt/wt) % and 43.3 (wt/wt) %. The maximum value was obtained at extraction time 50 min, biomass loading 1% and temperature 180 °C. The minimum value was obtained at extraction time 20 min, biomass loading 4.5 % and temperature 180 °C. The result showed that interaction of extraction time and biomass loading was necessary to have an increased amount of biomass solubilized.

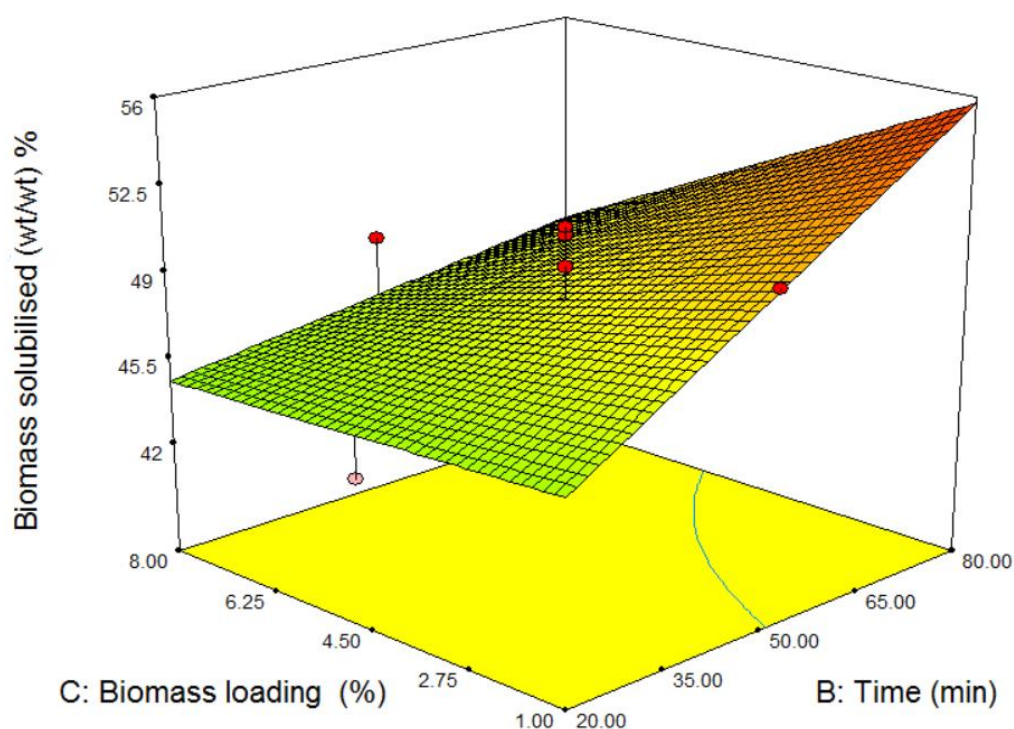


Figure 4-7: a 3D plot of the interaction effect of time and biomass loading on solubilisation at constant temperature 180°C

(B). Optimizing the biomass solubilisation and validating quadratic model

The quadratic model was also validated apart from using the statistical tools presented previously by comparing the predicted values of the biomass solubilised with that obtained from triplicate experiment under the same operating conditions of temperature, extraction time and biomass loading. The actual value of biomass solubilised was determined from same experiment used to optimise the lipid yield presented previously. The predicted biomass solubilised from the Software was 48.97 (wt/wt) % and the actual value gotten from the experiment was 34.2 ± 0.54 (wt/wt) %. Thus, a residual of 14.77 (wt/wt) % from the arithmetic difference between the predicted and actual value of biomass solubilised was not close to zero as had expected. The level of the validation of the model was low considering the outcome of this experiment but, the result was expected since the activated sludge used was from different sources, see Section 4.2.5.4. This was done due to the insufficient amount of biomass from the same source available to complete the experiments.

Generally, the interactive effects of the operating parameters impacted significantly on the biomass solubilisation with temperature having a greater influence. The biomass solubilisation was found to be directly proportional to the lipid yield at varied conditions of temperature, constant biomass loading and extraction time.

4.2.4.1.2 Investigating the impact of subcritical water mediated extraction on the lipid composition from activated sludge

(1). Lipid composition from untreated activated sludge

In order to obtain a basis for evaluating the impact of subcritical water treatment on lipid composition, the lipid extract from activated sludge was analysed using a gas chromatography (GC) equipped with flame ionization detector (FID) and DB-5HT column. This was done according to the method presented in Chapter 2.9.2.3. The lipids identified were triacylglyceride, diacylglyceride, monoglyceride, palmitoleic acid, palmitic acid, linoleic acid,

oleic acid, stearic acid, behenic acid, decanedioc acid, cetyl palmitate, cholesterol, phosphatidylcholine, phosphatidylinositol and phosphatidylserine, see Tables 4-11a and 4-11b. The profile of the fatty acids showed similarity with that obtained from FAME of the lipid extract, see Chapter 3.2.3.2.2 with palmitic acid been most predominant. Hence, the lipid profile could be used to evaluate the influence of subcritical water treatment. It is observed that unlike the previous analysis of lipid composition in activated sludge obtained from Finham STW (batch 2), the current results have shown that triacylglycerol (TAG) is present in the lipid extract from Minworth STW, see Section 3.3.3.2.1. It was suggested that the absence of TAG in the lipid extract from Finham STW (batch 2) could have resulted from hydrolysis of TAG to diacylglycerol (Revellame et al., 2012a), the reason for the presence in the lipid composition from Minworth STW, could be attributed to difference in the source of biomass. This is because, both lipid extracts were obtained from similar operating conditions, even when the biomass from Minworth STW was suggested to higher temperatures, some amounts of TAG were obtained at different operating parameters (Tables 4-11 and 4-12).

Table 4-11a: Lipid profile in the untreated activated sludge (Minworth STW, batch 1)

Run	Total amount of lipid (mg)	Amount of the lipid identified (mg)							
		TAG	DAG	MAG	PC	PS	PI	Cholesterol	Cetyl palmitate
1	50.32	0.18	0.10	0.04	0.03	0.02	0.06	0.05	0.04
2	88.80	0.32	0.17	0.07	0.05	0.04	0.10	0.08	0.07
3	50.32	0.18	0.10	0.04	0.03	0.02	0.06	0.05	0.04
4	11.10	0.04	0.02	0.01	0.01	0.004	0.01	0.01	0.01
5	11.10	0.04	0.02	0.01	0.01	0.004	0.01	0.01	0.01
6	88.80	0.32	0.17	0.07	0.05	0.04	0.10	0.08	0.07
7	50.32	0.18	0.10	0.04	0.03	0.02	0.06	0.05	0.04
8	88.80	0.32	0.17	0.07	0.05	0.04	0.10	0.08	0.07
9	11.10	0.04	0.02	0.01	0.01	0.004	0.01	0.01	0.01
10	88.80	0.32	0.17	0.07	0.05	0.04	0.10	0.08	0.07
11	11.10	0.04	0.02	0.01	0.01	0.004	0.01	0.01	0.01
12	50.32	0.18	0.10	0.04	0.03	0.02	0.06	0.05	0.04
14	50.32	0.18	0.10	0.04	0.03	0.02	0.06	0.05	0.04
15	11.10	0.04	0.02	0.01	0.01	0.004	0.01	0.01	0.01
16	50.32	0.18	0.10	0.04	0.03	0.02	0.06	0.05	0.04
17	50.32	0.18	0.10	0.04	0.03	0.02	0.06	0.05	0.04
18	50.32	0.18	0.10	0.04	0.03	0.02	0.06	0.05	0.04
19	50.32	0.18	0.10	0.04	0.03	0.02	0.06	0.05	0.04
20	88.80	0.32	0.17	0.07	0.05	0.04	0.10	0.08	0.07

TAG = triacylglycerol, DAG = diacylglycerol, MAG = monoacylglycerol, PC = L – α - phosphatidylcholine, PS = 1,2-diacyl-Sn-glycero-3-phospho-L-serine and PI = L – α – phosphatidylinositol

Table 4-11b: Lipid profile in the untreated activated sludge (Minworth STW, batch 1) (contd)

S/N	Total amount of lipid (mg)	Amount of the lipid identified (mg)						
		Palmit-oleic acid	Palmitic acid	Linoleic acid	Oleic acid	Stearic acid	Behenic acid	Decane-dioc acid
1	50.32	0.26	4.71	0.77	0.74	0.77	0.15	0.19
2	88.80	0.45	8.31	1.36	1.31	1.36	0.26	0.33
3	50.32	0.26	4.71	0.77	0.74	0.77	0.15	0.19
4	11.10	0.06	1.04	0.17	0.16	0.17	0.03	0.04
5	11.10	0.06	1.04	0.17	0.16	0.17	0.03	0.04
6	88.80	0.45	8.31	1.36	1.31	1.36	0.26	0.33
7	50.32	0.26	4.71	0.77	0.74	0.77	0.15	0.19
8	88.80	0.45	8.31	1.36	1.31	1.36	0.26	0.33
9	11.10	0.06	1.04	0.77	0.16	0.77	0.03	0.04
10	88.80	0.45	8.31	1.36	1.31	1.36	0.26	0.33
11	11.10	0.06	1.04	0.17	0.16	0.17	0.03	0.04
12	50.32	0.26	4.71	0.77	0.74	0.77	0.15	0.19
14	50.32	0.26	4.71	0.77	0.74	0.77	0.15	0.19
15	11.10	0.06	1.04	0.17	0.16	0.17	0.03	0.04
16	50.32	0.26	4.71	0.77	0.74	0.77	0.15	0.19
17	50.32	0.26	4.71	0.77	0.74	0.77	0.15	0.19
18	50.32	0.26	4.71	0.77	0.74	0.77	0.15	0.19
19	50.32	0.26	4.71	0.77	0.74	0.77	0.15	0.19
20	88.80	0.45	8.31	1.36	1.31	1.36	0.26	0.33

(2) Investigating the effect of subcritical water treatment on lipid composition from subcritical on activated sludge (Minworth STW, batch 1) using FCCCD

The extract obtained from the FCCCD subcritical water mediated extraction on activated sludge was analysed, in order to study the impact of the treatment on the lipid composition. The analysis was done using the method described in Chapter 2.9.2.3. The lipid composition was similar to those obtained from the untreated activated sludge and are presented in Tables 4-12a and 4-12b. This shows that palmitic acid was in higher proportion compared to other lipids under the same conditions of temperature, extraction time and biomass loading. The arithmetic difference (not presented) between the subcritical water treated activated sludge and

that untreated, indicated that there was a predominant significant impact of subcritical water treatment on the lipid composition, under the varying operating conditions. This might be due to increase in biomass solubilisation and the catalytic effect of water under subcritical conditions, as have been explained in Section 4.2.7.2. Tsigie et al. (2012a) also observed increase in amount of mono-, di- and triacylglycerol and attributed that of wax ester as increase in its dissolution in water when subjected to subcritical water treatment. Although, diacylglycerol (DAG) is found in trace amount in both fresh plant and animal tissues, its large presence in the lipid extract is attributed to the hydrolysis of triacylglycerol (TAG) and phospholipids (Christie, 2011a). For example, the hydrolysis of phosphatidylinositol by phospholipase C gives rise to 1,2-diacyl-sn-glycerols (Christie, 2011a). DAG can be used in the production of soap, detergents, plastic and renewable energy (Ravellame et al., 2012a).

Table 4-12a: Lipid profile from the FCCCD subcritical water mediated extraction on activated sludge (Minworth STW, batch 1)

Run	Process conditions			Total amount of lipid (mg)	Amount of the lipid identified (mg)							
	Temperature (°C)	Time (min)	Bio-mass loading (%)		TAG	DAG	MAG	PC	PS	PI	Cholesterol	Cetyl Palmi-tate
1	180	50	4.5	66.80	0.58	2.40	0.07	0.21	0.45	0.07	0.08	0.05
2	280	20	8.0	90.20	0.39	2.11	0.05	0.18	0.34	0.16	0.03	0.06
3	180	50	4.5	113.50	1.15	3.01	0.12	0.30	0.82	0.30	0.19	0.14
4	280	80	1.0	39.50	0.42	1.76	0.12	0.14	0.32	0.04	0.07	0.06
5	280	20	1.0	53.70	0.64	2.59	0.11	0.16	0.45	0.05	0.05	0.03
6	80	20	8.0	91.20	0.28	2.33	0.16	0.22	0.36	0.17	0.09	0.08
7	180	50	4.5	101.70	0.83	4.51	0.16	0.36	1.04	0.16	0.11	0.08
8	80	80	8.0	84.70	0.54	2.80	0.23	0.69	0.91	0.13	0.47	0.35
9	80	20	1.0	61.50	0.65	2.95	0.14	0.15	0.53	0.07	0.05	0.06
10	280	80	8.0	220.50	0.82	3.66	0.37	0.91	1.25	0.13	0.22	0.14
11	80	80	1.0	45.40	0.63	2.44	0.08	0.05	0.54	0.05	0.04	0.08
12	180	50	4.5	89.00	0.87	2.81	0.44	0.57	0.83	0.11	0.21	0.62
14	80	50	4.5	71.90	0.34	4.40	0.06	0.18	0.55	0.21	0.14	0.73
15	180	50	1.0	49.20	0.21	3.70	0.05	0.12	0.31	0.10	0.04	0.10
16	280	50	4.5	139.10	0.35	6.76	0.22	0.59	0.40	0.16	0.09	0.19
17	180	50	4.5	82.20	0.21	2.88	0.11	0.07	0.31	0.24	0.16	0.99
18	180	20	4.5	93.30	0.19	3.29	0.12	0.08	0.51	0.26	0.25	0.20
19	180	80	4.5	23.76	0.29	2.27	0.11	0.16	0.49	0.08	0.06	0.12
20	180	50	8.0	30.84	0.44	2.83	0.32	0.34	0.41	0.49	0.16	0.17

Table 4-12b: Lipid profile from the FCCCD subcritical water mediated extraction on activated sludge (Minworth STW, batch 1) (contd)

Run	Process conditions			Total amount of lipid (mg)	Amount of the lipid identified (mg)						
	Temperature (°C)	Time (min)	Bio-mass loading (%)		Palmit-oleic acid	Palmitic acid	Linoleic acid	Oleic acid	Stearic acid	Behenic acid	Decane-dioc acid
1	180	50	4.5	66.80	0.46	4.51	1.04	0.84	0.76	3.81	1.36
2	280	20	8.0	90.20	0.48	4.17	0.63	0.53	0.54	3.12	0.28
3	180	50	4.5	113.50	2.19	8.29	2.24	1.92	1.87	5.35	0.95
4	280	80	1.0	39.50	0.46	2.67	0.70	0.96	0.60	3.50	1.02
5	280	20	1.0	53.70	0.47	3.70	0.92	0.54	0.68	3.69	0.16
6	80	20	8.0	91.20	1.09	4.73	0.49	1.04	0.55	5.26	4.34
7	180	50	4.5	101.70	0.59	4.96	1.27	1.14	0.71	4.94	0.31
8	80	80	8.0	84.70	0.80	7.29	1.08	1.01	1.99	6.18	5.23
9	80	20	1.0	61.50	0.18	1.98	0.85	0.46	0.44	3.71	0.18
10	280	80	8.0	220.50	2.42	11.66	1.33	2.38	1.16	5.79	0.48
11	80	80	1.0	45.40	0.11	1.77	0.83	0.23	0.45	3.58	0.06
12	180	50	4.5	89.00	0.86	6.61	1.62	1.38	1.52	4.28	0.25
14	80	50	4.5	71.90	0.53	4.31	0.96	0.93	1.06	6.71	10.35
15	180	50	1.0	49.20	0.42	2.76	0.46	0.59	0.65	6.33	9.75
16	280	50	4.5	139.10	1.83	8.54	2.35	2.40	1.56	7.61	7.34
17	180	50	4.5	82.20	0.46	5.96	0.89	1.00	1.07	5.43	7.40
18	180	20	4.5	93.30	0.38	6.61	1.08	1.45	1.55	8.62	11.61
19	180	80	4.5	23.76	0.40	5.78	2.15	2.17	1.36	3.62	2.92
20	180	50	8.0	30.84	0.81	9.01	2.50	1.83	1.91	4.19	3.06

The quantities of the lipid composition: fatty acid, acylglycerol, sterol and phospholipids obtained from GC analysis of the extract was analysed using RSM in order to determine optimum operating conditions which had greater impact on them during the subcritical water treatment. The effect of single and interaction of operating parameters were evaluated. The amounts of the lipid obtained from GC were inputted into the Face Centred Central Composite Design (FCCCD) in Design Expert Software 7.0 2005 and then analysed (Table 4-12a and b). The results of fatty acid (palmitoleic acid, palmitic acid, and oleic acid), acylglycerol (monoacyl glycerol), sterol (cholesterol), and phospholipids (phosphatidylcholine (PC) and phosphatidylserine (PS)) are presented below:

(1.) *Fatty acids*

The fatty acids: palmitoleic acid, palmitic acid and oleic acid were modelled using FCCCD to determine the main and interaction effects on the quantity recovered. The analysis of variance (ANOVA) revealed that reduced quadratic models best described the processes, see Equation 4-3. The p-values (Prob > F) were 0.0005, 0.0022 and 0.0219 for palmitoleic acid, palmitic acid and oleic acid, respectively and these showed that the models are significant. The Lack of Fits values are insignificant and these included 0.3985, 0.7404 and 0.6339 for palmitoleic acid, palmitic acid and oleic acid, respectively. The models are presented in terms of actual factors temperature (A), time (B) and biomass loading (C) and the results show that they can be used to navigate the design spaces. The model graphs are presented in terms of single factor and

$$\begin{aligned} (a). \text{Palmitoleic acid (mg)} &= 1.10261 - 0.015158A + 6.15140E - 004 * B - \\ &\quad 0.021558 * C + 9.71429E - 004 * AC + 4.46851E - 005 * A^2 \quad (R^2 = 0.8867) \\ (b). \text{Palmitic acid (mg)} &= 0.40527 + 0.01660 * A + 0.016870 * B + 0.57646 * C + \\ &\quad 0.013440 * BC - 5.07521E - 004 * B^2 - 0.062593 * C^2 \quad (R^2 = 0.8032) \\ (c). \text{Oleic acid} &= 0.83904 - 2.13083E - 003 * A - 0.032564 * B + 0.36608 * \\ &\quad + 1.05417E - 004 * AB + 1.94048E - 003 * BC + 1.39573E - 004 * B^2 - \\ &\quad 0.038725C^2 \quad (R^2 = 0.7423) \end{aligned}$$

Equation 4-3

interaction effects of the operating parameters on the quantities of fatty acid recovered. The interaction effect of biomass loading and temperature at constant extraction time of 50 min gave palmitoleic acid of 0.11 to 2.42 mg (Figure 4-8). The results show that single factors temperature and biomass loading and their interaction significantly impacted on the amount of palmitoleic acid recovered from the extraction process. Comparatively, subcritical water treatment helped to increase the amount of palmitoleic acid from a maximum of 0.45mg

untreated to 2.42 mg, thereby rendering itself as a potential for competitive oleochemical and bioenergy (biodiesel and renewable) production.

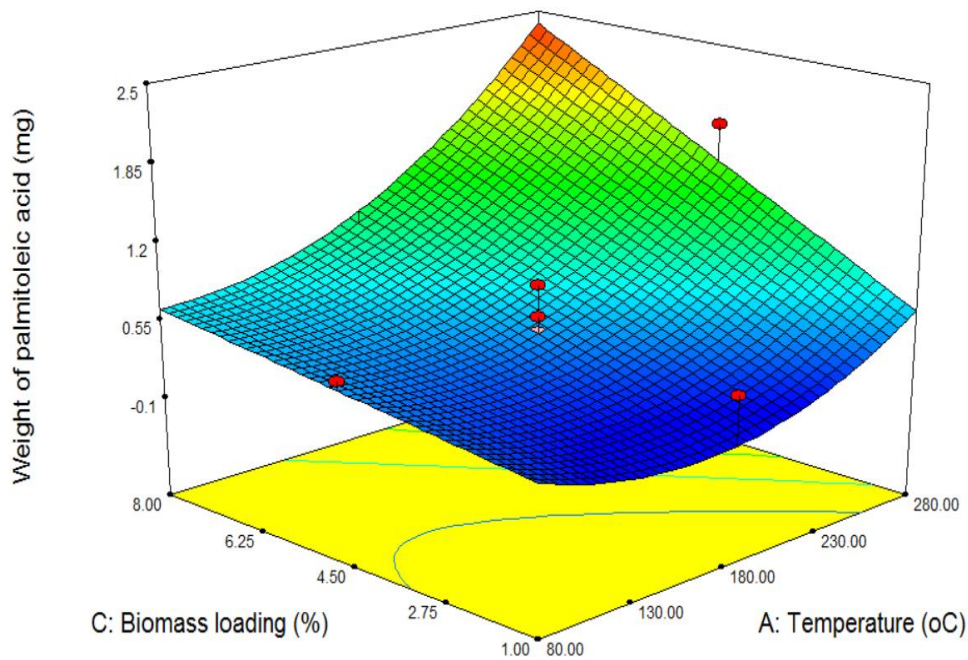


Figure 4-8: interaction effect of biomass loading and temperature at 50 min on the amount of palmitoleic acid extracted from activated sludge under subcritical water extraction

Furthermore, the palmitic acid response model (Equation 4-3b) was used to navigate the design space to predict the impact of single factors and interaction effect of temperature, extraction time and biomass loading on quantity of palmitic acid recovered from subcritical water mediated extraction. The interaction effect of extraction time and biomass loading produced a minimum of 1.77 mg and a maximum of 11.66 mg of palmitic acid at 180°C. The results show that the interaction of extraction time and biomass loading had a significant impact on the quantity of palmitic acid recovered post subcritical water treatment (Figure 4-9).

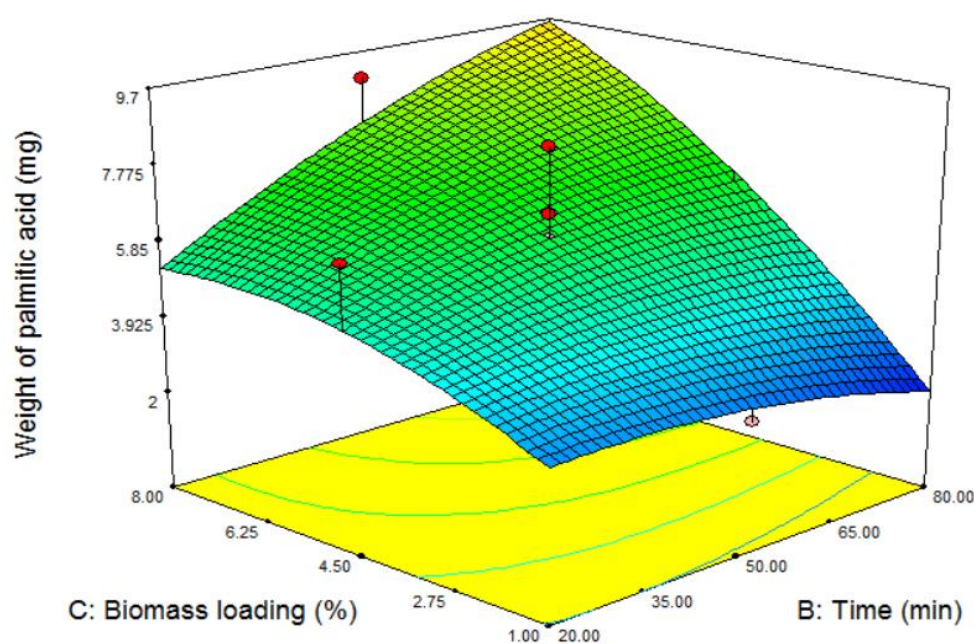


Figure 4-9: interaction effect of extraction time and biomass loading at 180°C on the amount of palmitic acid extracted from activated sludge under subcritical water extraction

The quadratic model (Equation 4-3c) was applied to predict the impact of main and interaction effects of temperature, extraction time and biomass loading on quantity of oleic acid recovered from subcritical water mediated extraction. The interaction effect of temperature - extraction time at 4.5%, and biomass loading and extraction time – biomass loading at 180°C are demonstrated in Figures 4-10 and 4-11, these gave rise to oleic acid of 0.23 to 2.4 mg.

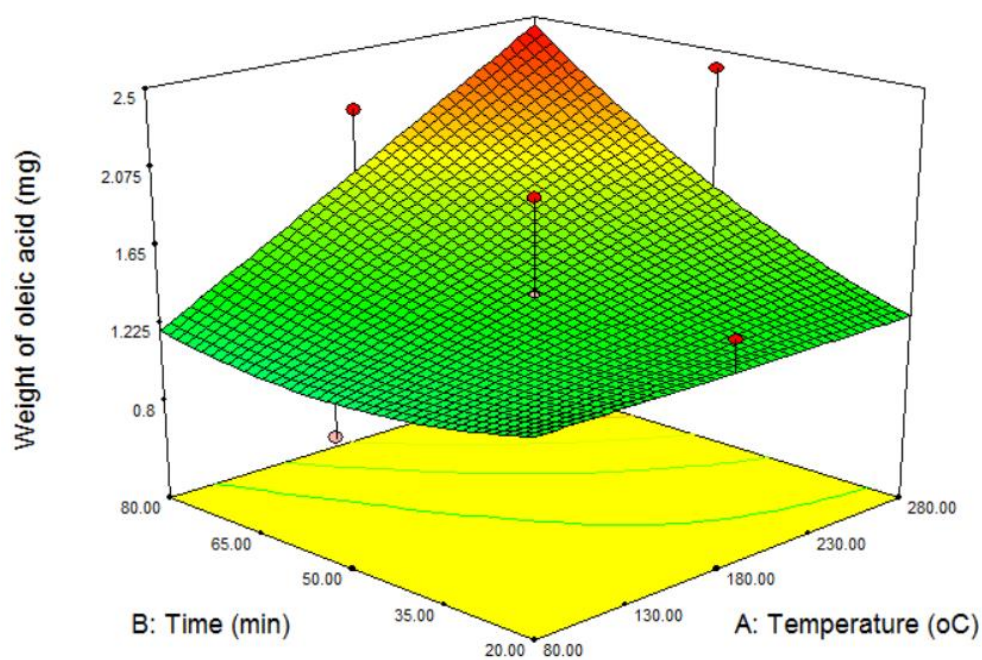


Figure 4-10: interaction effect of extraction time and temperature at 4.5% biomass loading on the amount of oleic acid extracted from activated sludge under subcritical water extraction

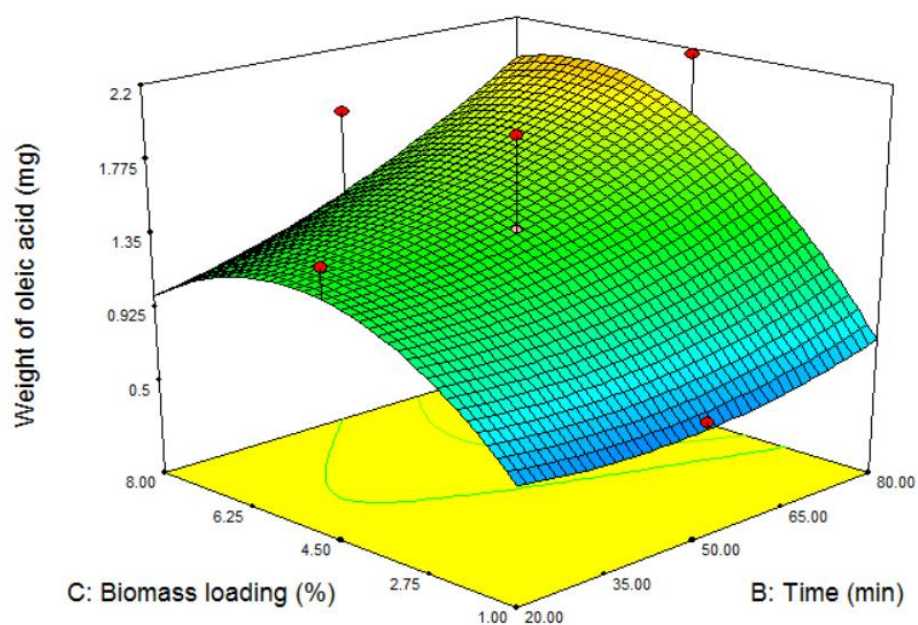


Figure 4-11: interaction effects of extraction time and biomass loading at 180°C on the amount of oleic acid extracted from activated sludge under subcritical water extraction

From these results, it was observed that single factors of temperature and biomass loading with their interaction and extraction time – biomass loading had the most significant effect on the amount of oleic acid recovered.

(2). Acylglycerol

The impact of operating parameters: temperature, extraction time and biomass loading on the amount of monoglyceride (MAG) (response) was evaluated. Multiple regression analysis was carried out on the experimental data using Design Expert 7.0 2005 to obtain a relationship between the response and operating parameters. The quadratic model obtained is presented in terms of actual parameters: Temperature (A), extraction time (B) and biomass loading (C) (Equation 4-4). Analysis of variance (ANOVA) revealed that the model was significant and Lack of Fit was insignificant based on the p-values of 0.0150 and 0.3902, respectively. ANOVA also showed that biomass loading and its interaction with extraction time were the significant model terms.

$$\text{Monoglyceride (mg)} = 0.27 - 4.67\text{E} - 004 * A - 3.66\text{E} - 003 * B - 0.04 * C + 1.33\text{E} - 005 * AB + 5.24\text{E} - 004 * BC + 3.98\text{E} - 003 * C^2$$

Equation 4-4

The determination coefficient (R^2) of 0.7413 indicated that the model can be used to explain 74.13% variability in the response (monoglyceride) parameter. The model graphs are presented in Figures 4-12 and 4-13, and they showed the relationships between the independent parameters and their interactions. The interactions of temperature and extraction time at 4.5% biomass loading and extraction time and biomass loading at 180°C resulted to increase in monoglyceride from 0.05 mg to 0.37 mg, respectively (Figures 4-12 and 4-13).

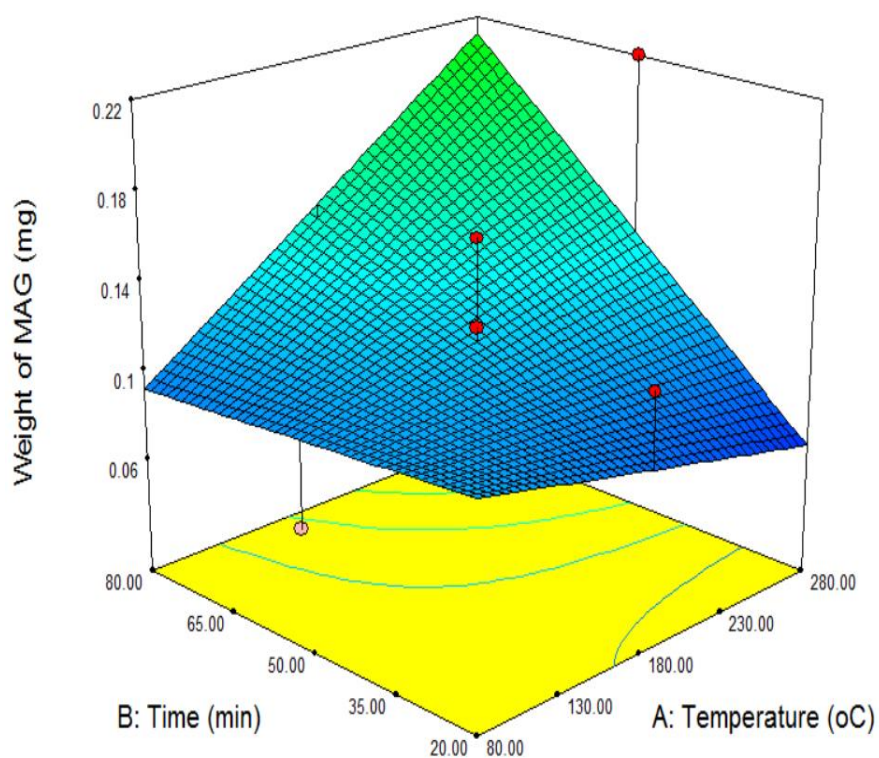


Figure 4-12: interaction effect of temperature and extraction time at 4.5% biomass loading on the amount of monoglyceride extracted from activated sludge under subcritical water extraction

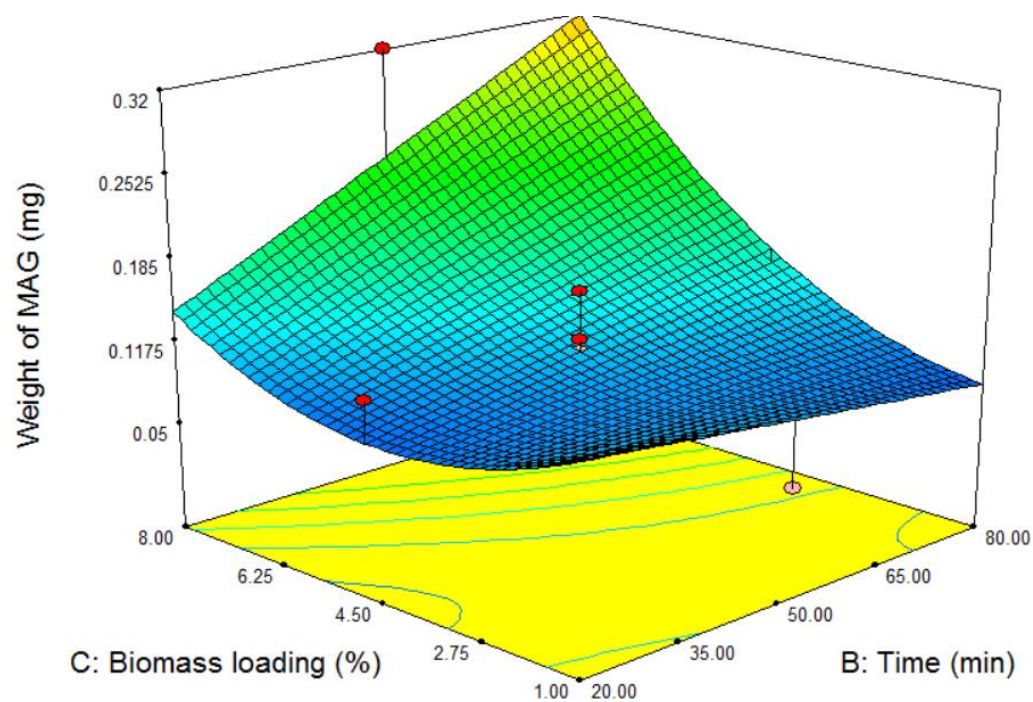


Figure 4-13: Interaction effect of extraction time and biomass loading at 180°C on the amount of monoglyceride acid extracted from activated sludge under subcritical water extraction

(3). Sterol

The impact of independent parameters temperature, extraction time and biomass loading and their interaction effects on quantity of cholesterol response from GC analysis was evaluated. A quadratic model obtained was suggested as the best fitting for the experimental data (Equation 4-5). The analysis of variance (ANOVA) of the data resulted to a p – value of 0.0321 and Lack of Fit of 0.3348. These results show that the model is significant and Lack of Fit is insignificant, indicating that it can be used to assess the impact of the operating parameters on the amount of cholesterol recovered. The determination coefficient (R^2) of the model was 0.6617 showing that it can be used to explain 66.17% variability in the quantity of cholesterol. The model was expressed in terms of actual factors with A, B, C representing temperature, extraction time and biomass loading, respectively.

$$\text{Cholesterol (mg)} = 0.05 + 2.16E - 004 * A - 1.7E - 003 * B + 0.03 * C - 1.21E - 004 * AC + 6.67E - 004 * BC - 2.20E - 003 * C^2 \quad \text{Equation 4-5}$$

The interaction effects obtained using the model are presented in the 3D plots shown in Figures 4-14 and 4-15. The interaction of temperature - biomass loading at extraction time of 50 min and extraction time – biomass loading at temperature of 180°C resulted to an increase in the amount of cholesterol from 0.03 to 0.47 mg (Figures 4-14 and 4-15). These results show that biomass loading and its interaction effect with extraction time had greatest impact on the amount of cholesterol obtained compared to other parameters. Thus, the model can be used to navigate the design space.

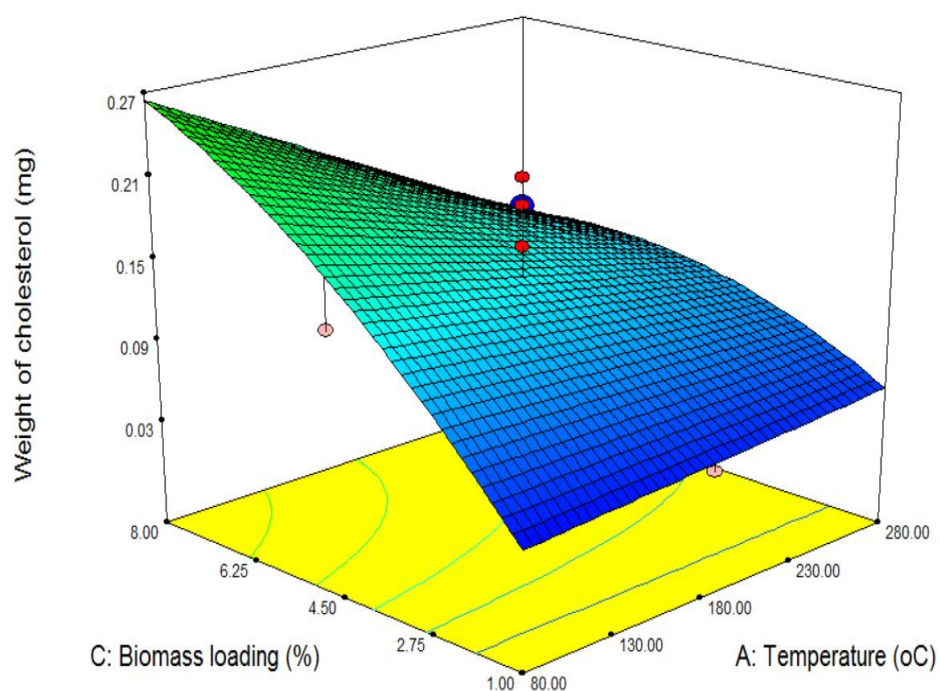


Figure 4-14: interaction effect of temperature and biomass loading at 50 min on the amount of cholesterol extracted from activated sludge under subcritical water extraction

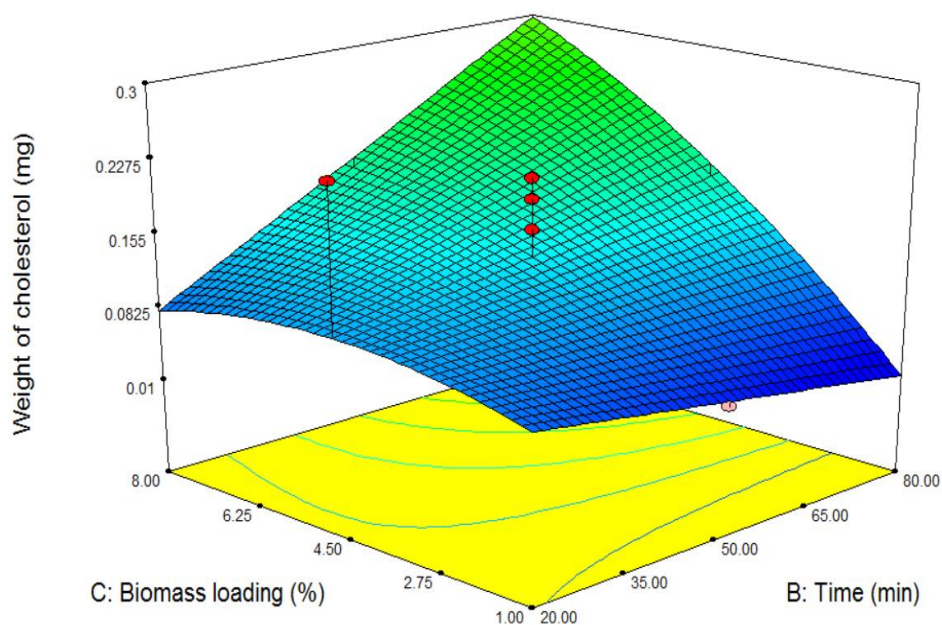


Figure 4-15: interaction effect of extraction time and biomass loading at 180°C on the amount of cholesterol extracted from activated sludge under subcritical water extraction

(4). Phospholipids

(i). Phosphatidylcholine (PC)

FCCCD was used to evaluate the operating parameters: temperature, extraction time and biomass loading, to understand how they influence the amount of PC (response) obtained from GC analysis post subcritical water mediated lipid extraction from activated sludge (Table 4-12a). Upon multiple regression analysis, a quadratic model relating the response and independent parameters was obtained. The model was expressed in terms of actual factors temperature (A), extraction time (B) and biomass loading (C) (Equation 4-6). ANOVA revealed that the F-values 4.99 and 0.76 indicated that the model was significant and Lack of Fit was insignificant. The “Prob > F” values B (0.0278), C (0.0037) and BC (0.0087) less than 0.05 showed that they are significant model terms. Whereas model terms A (0.1534), AB (0.4126), AC (0.8443), A^2 (0.1622) and B^2 (0.1105) are not significant as their “Prob > F” values are greater than 0.1.

$$PC (mg) = 0.25 - 4.56E - 003 * A + 0.01 * B - 0.03 * C + 1.42E - 005 * AB + 2.86E - 005 * AC + 1.57E - 003 * BC + 1.22E - 005 * A^2 - 1.58E - 004 * B^2$$

Equation 4-6

The coefficient of determination (R^2) of 0.8162 showed that the model can be used to explain 81.62% discrepancies in the response factor (Gunawan et al., 2014). The model graphs are shown in the third-dimensional (3D) representation of the relationship between the response and operating parameters with their interaction (Figures 4-16 and 4-17). The interactions of temperature with extraction time at biomass loading of 4.5% and temperature with biomass loading at 50 min resulted to a PC from 0.05 mg to 0.91mg, respectively (Figures 4-16 and 4-17). The results show that operating parameters temperature, extraction time, biomass loading and the interactions between extraction time and biomass loading had great impacts on the amount of PC extracted, see also (Tables 4-11a and 4-12a).

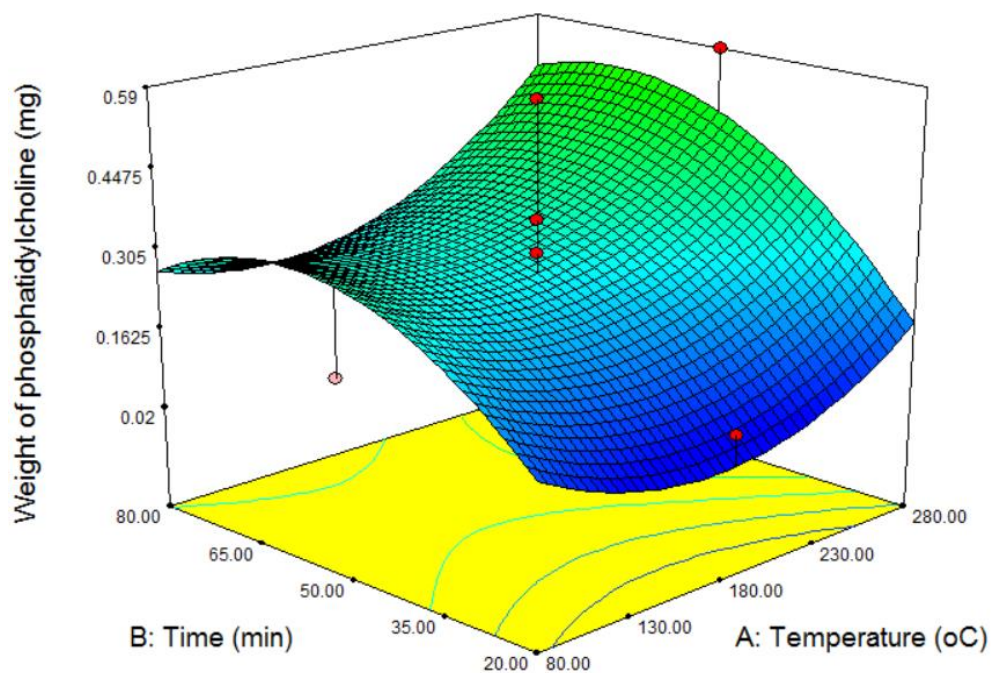
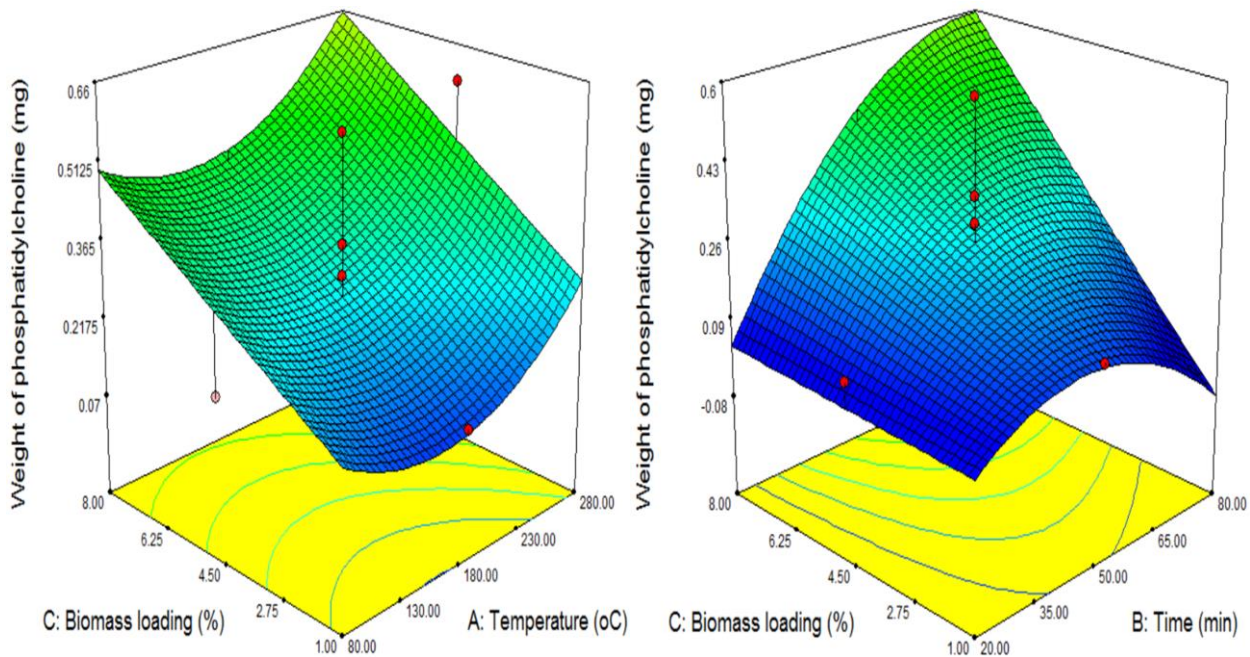


Figure 4-16: interaction effect of temperature and extraction time using 4.5% biomass loading on the amount of phosphatidylcholine extracted from activated sludge under subcritical water extraction



(a). Interaction effect of temperature and biomass loading at 50 min

(b). Interaction effect of extraction time and biomass loading at 180 °C

Figure 4-17: interaction effects of the operating parameters on the amount of phosphatidylcholine extracted from activated sludge under subcritical water extraction

(ii). Phosphatidylserine (PS)

The effects of factors: temperature, extraction time and biomass loading and their interactions on the amount of PS recovered post subcritical treatment of activated sludge were investigated. Multiple regression was carried out on the experimental data (Table 4-12a). A quadratic model was obtained as the best fitting for the experimental data, in terms of the actual factors temperature (A), extraction time (B) and biomass loading (C) (Equation 4-7). The analysis of variance (ANOVA) revealed that the model was significant as the F-value was 3.40. The significant model terms were B and BC, with p-values (Prob > F) of 0.0426 and 0.0111, respectively, which were less than 0.05, as required. The Lack of Fit was insignificant in relation to pure error as the F-value was 0.34, indicating that the model is a good fit and suitable for describing the experimental data. The coefficient of determination (R^2) was 0.7039,

indicating that the model can be used to describe 70.39% variation in the response factor (Gunawan et al., 2014).

$$PS (mg) = 0.59 - 1.13E - 003A - 2.54E - 003B + 0.01C + 2.21E - 004AC + 1.88E - 003BC - 1.52E - 005B^2 - 0.01C^2 \quad \text{Equation 4-7}$$

The 3D model graphs representing the effects of factors with their interaction on the amount of PS recovered are presented in Figures 4-18 and 4-19. The interaction of temperature and biomass loading at 50 min, and that between extraction time and biomass loading at 180°C yielded a minimum and maximum of 0.31 mg and 1.25 mg, respectively, of PS (Figures 4-18 and 4-19).

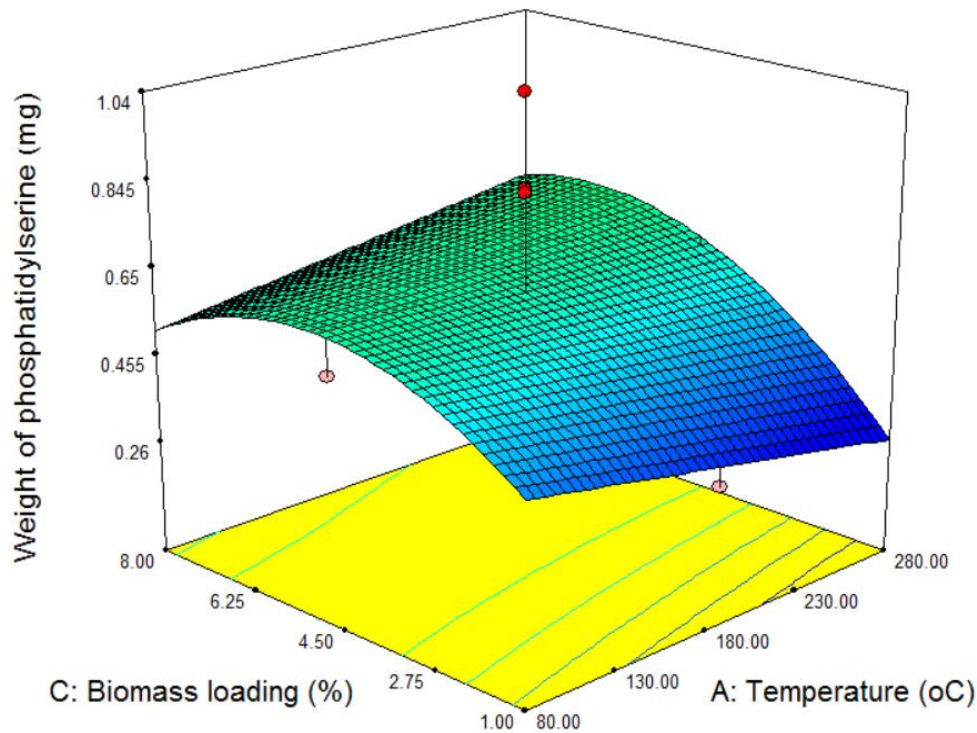


Figure 4-18: interaction effect of temperature and biomass loading at the extraction time of 50 min on the amount of phosphatidylserine extracted from activated sludge under subcritical water extraction

These results and those shown in Tables 4-11a and 4-12a show that subcritical water treatment had an impact on the amount of PS recovered with extraction time and its interaction with biomass loading been the significant factors.

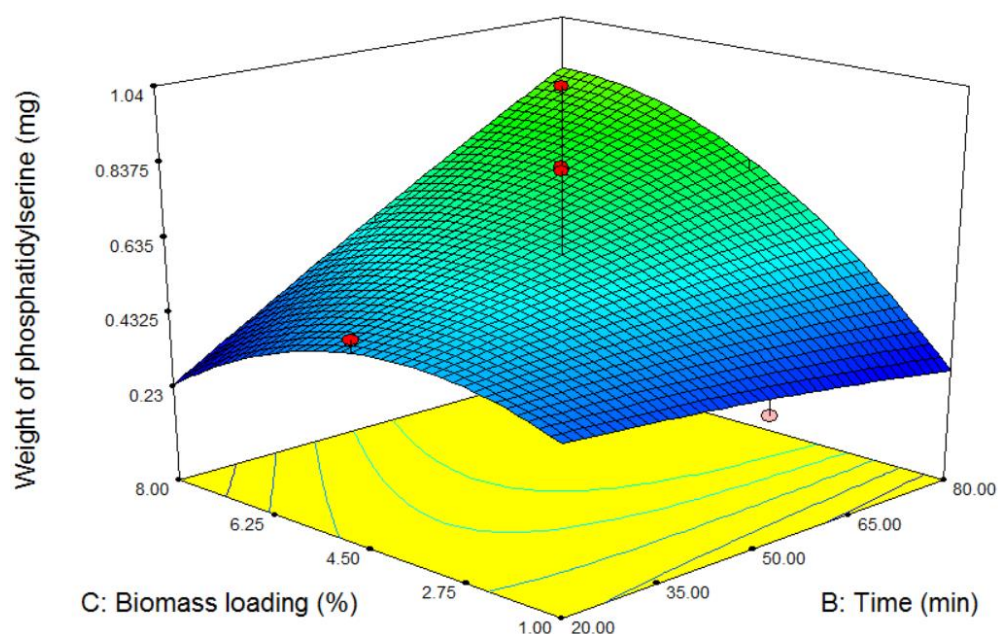


Figure 4-19: Interaction effects of extraction time and biomass loading on the amount of phosphatidylserine extracted from activated sludge under subcritical water extraction at constant temperature 180°C.

Generally, the impacts of operating parameters used during the subcritical water treatment on the composition of lipids: fatty acid (palmitoleic acid, palmitic acid and oleic acid), acylglycerol (monoacylglycerol), sterol (cholesterol) and phospholipids (PC and PS) are discussed below.

(1). Effect of temperature on lipid composition from activated sludge

The impact of temperature 80, 180 and 280°C was investigated using FCCCD (Design Expert 7.0 2005) at constant extraction time 50 min and biomass loading 4.5%. The study revealed that temperature significantly impacted on fatty acids and this is in agreement with the findings by Tsigie et al. (2012a). The quantity of fatty acids and monoglycerol increased

significantly compared to other lipids with increase in temperature. That of Cholesterol and PS decreased with increase in temperature as predicted by the quadratic models. The same trend of decreased was also observed in triacylglycerol, diacylglycerol, cetyl palmitate and PI (Table 4-12a). The increase in the quantity of fatty acids and monoacylglycerol is attributed to the hydrolysis of phospholipids, wax ester, sterol, di- and tri acylglycerol at high temperature 280°C. These results were corroborated by those obtained by Nguyen et al. (2013) in which they reported an increase in fatty acid at 175°C for 15 min. According to them, hydrolysis of acylglycerol, glycolipids, wax esters which was evidenced in their decrease after treatment, resulted to an increase in free fatty acid.

(2). Effect of extraction time on lipid composition from activated sludge

The investigation on impact of extraction time (20, 50 and 80 min) on lipid composition at constant temperature 180°C and biomass loading 4.5% has been presented. The results revealed that 20 min was the optimum extraction time for the extraction of palmitic acid, monoacylglycerol, and cholesterol. A further increase in time to 50 min was required to obtain a maximum amount of palmitoleic acid, PC and PS and to 80 min to extract the highest quantity of oleic acid. The reduction in the amount of lipid after the optimum extraction time is attributed to hydrothermal degradation.

(3). Effect of biomass loading on lipid composition from activated sludge

The impact of biomass loading (1, 4.5 and 8%) on lipid profile was studied at constant temperature 180°C and extraction 50 min. We found that it was a significant parameter in the extraction of palmitoleic acid, palmitic acid, oleic acid, monoacylglycerol, cholesterol and PC, given that increase in biomass loading resulted to a corresponding increase in those lipids. This effect produced a maximum increase in lipid profile at 8%, except for PS where 4.5% biomass loading was the optimum condition.

(4). *Effect of interaction of operating parameters on lipid composition from activated sludge*

Studies were carried out on the impact of interaction of operating parameters: temperature (A), extraction time (B) and biomass loading (C) on lipid composition based on the model prediction. The interaction effects from temperature-biomass loading (AC), temperature-extraction time (AB) and extraction time-biomass loading (BC) were found to significantly affect the lipid composition. With AC having a great impact on the amount of palmitoleic acid (Figures 4-8), AB on oleic acid (Figure 4-10) and BC on all the lipids, except palmitoleic acid. The increase in the proportion of lipid composition after treatment with subcritical water was expected since distinct parameters showed a significant impact.

4.3 CONCLUSIONS

Following the subcritical water mediated extraction of lipid from activated sludge and subsequent optimisation of the total lipid, composition and biomass solubilisation, the under listed conclusions are made:

1. That water a benign solvent under subcritical condition increased both the lipid yield and composition, from activated sludge.
2. That the lipid yield 3.97 ± 2.34 (wt/wt) % obtained using methanol (Soxhlet apparatus) increased 4.6 times when activated sludge was treated with subcritical water at 280°C, 5 % biomass loading and extraction time of 40 min.
3. That upon optimisation using Response Surface Methodology (RSM), subcritical treatment on activated sludge at 80°C using 1% biomass loading for 20 min gave an optimum lipid yield of 41.0 (wt/wt) % resulting to 454.1(wt/wt) % increase when compared to 7.4 (wt/wt) % obtained from Folch et al. (1957).

4. That upon optimisation and subcritical water treatment on activated sludge, biomass solubilisation showed a direct proportional relationship with lipid yield
5. That increased amount of triacylglyceride, diacylglyceride, monoglyceride, palmitoleic acid, palmitic acid, linoleic acid, oleic acid, stearic acid, behenic acid, decanedioc acid, cetyl palmitate, cholesterol, phosphatidylcholine, phosphatidylinositol and phosphatidylserine post subcritical water treatment provides a potential cheap feedstock for the oleochemical industries
6. That increased lipid yield would help to increase feedstock availability for bioenergy (biodiesel and renewable diesel) production at a cheaper cost, since the extraction is carried out at minimum operating conditions of temperature 80°C, extraction time 20 min and biomass loading 1%.

5 CHAPTER 5 PRODUCTION OF

RENEWABLE DIESEL

5.1 INTRODUCTION

Biodiesel is considered as one of the alternative sources of fuels which has the potential to displace the conventional fossil fuels, see Chapters 1 and 3. Although, it is renewable, biodegradable, has good lubricity, its energy density resembling that of petroleum diesel, lower particulate exhaust emission and higher flash point than petroleum diesel (Fu et al., 2010, Knothe, 2005, B. Liu and Zhao, 2007, Meng et al., 2008), its drawbacks include poor cold flow, low energy density and relatively high cost of production (Wang et al., 2012). Most of these drawbacks can be minimised through the use of renewable diesel. This kind of diesel has better quality in terms of cetane, cloud point, oxidative stability and cold flow, and can easily be adapted to the existing refinery processes and transportation facilities, thereby, reducing the cost of production (Kalnes et al., 2007). It is commonly produced by hydrotreating of fats and oils and by deoxygenation of free fatty acids (Do et al., 2009, Kalnes et al., 2007, Snare et al., 2006) in the presence of catalyst such as $\text{CoMo/Al}_2\text{O}_3$ and $\text{NiMo/Al}_2\text{O}_3$ and high partial pressure hydrogen (Lestari et al., 2009, Smith et al., 2009), see Chapter 1. The problem with this method is that hydrogen is required. This is because, hydrogen increases the cost of production and makes the entire process dependent on fossil fuel, since, it is produced mainly from steam reforming of natural gas (Fu et al., 2011a). Based on this problem, decarboxylation of fats/oils or free fatty acids is being considered. It does not require the use of hydrogen and produces hydrocarbons with higher energy density (Fu et al., 2011a). Most previous works on

decarboxylation were done in organic medium containing substances such as dodecane and mesitylene (Fu et al., 2011a). These substances also increase the cost of production.

However, in order to reduce cost, the current investigation is geared toward assessing the potential of producing renewable diesel/hydrocarbons from lipid extract derived from activated sludge, using catalytic hydrothermal decarboxylation without the addition of hydrogen, see Chapters 1, 2, and 4. Palmitic and oleic acids were used as model fatty acids to produce hydrocarbons/renewable diesel, using hydrothermal decarboxylation, at varied temperature and residence time. The catalysts used were palladium on activated carbon (Pd/C) and platinum on activated carbon (Pt/C). The experiments were conducted as described in Section 2.7.4. The uncertainties experienced are reported in terms of standard error of the mean, as shown in the Figures.

5.2 RESULTS AND DISCUSSIONS

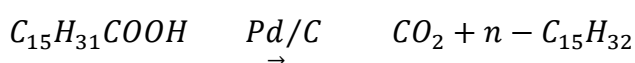
5.2.1 Hydrothermal decarboxylation of palmitic acid

Palmitic acid was used as a model fatty acid to carry out hydrothermal decarboxylation reaction using palladium and platinum on activated carbons as catalysts. This was done to study the effects of the operating parameters: temperature and residence time on the conversion of palmitic acid, and yield and selectivity of the hydrocarbon products, which would aid understanding of a similar reaction using lipid derived from activated sludge as the feedstock.

5.2.1.1 Hydrothermal decarboxylation of palmitic acid using palladium on activated carbon (Pd/C) catalyst

This was conducted in order to evaluate the potential of producing hydrocarbons, especially pentadecane from palmitic acid, using hydrothermal catalytic processes without the addition of hydrogen. To achieve this, the effects of Pd/C catalyst was studied at varied temperatures and

residence times while keeping other parameters constant. The details are presented in Sections 5.2.1.1.1 and 5.2.1.1.2. The reaction scheme is presented below for the selective decarboxylation of palmitic acid ($C_{15}H_{31}COOH$) to pentadecane ($n - C_{15}H_{32}$) using palladium on activated carbon (Pd/C) catalyst. The by-products may include 1-tetracosanol, decylcyclopentane, 4-methyl tetradecane, tridecane, dodecane, undecane, nonane, octane and hexadecane, as suggested by Fu et al. (2010). In addition to these products, we also identified heptadecane as a secondary product.



Scheme 5-1: illustration of the decarboxylation of palmitic acid in the presence of Pd/C

5.2.1.1.1 Effect of temperature on hydrothermal decarboxylation of palmitic acid using palladium on activated carbon (Pd/C) catalyst

Experiments were carried out according to the method described in Section 2.7.4, in order to evaluate the impact of temperatures (290, 300 and 330 °C) on hydrothermal decarboxylation of palmitic acid (100 mg) at constant residence time of 4 h using 20 mg of Pd/C catalyst and 15 mL of water in a 22.5 mL mini reactor. The results showed maximum yields of 15.6 % pentadecane and 2.1% of heptadecane at 290°C and minimum values of 4.6 % and 1.5% at 330°C, respectively (Figure 5-1). Thus, a temperature of 290 °C was required to produce a maximum yield of pentadecane (15.6 %) and 2.1 % of heptadecane after 4 h of hydrothermal decarboxylation of palmitic acid. Figure 5-2 shows the impact of temperature on the conversion of palmitic acid and selectivity of the hydrocarbon products. The conversion was lowest (59.5%) at 290°C while it was highest (76%) at 300°C. The highest conversion of palmitic acid (76 %), recorded at 300 °C with a lower molar yield (8.1 %) compared to that obtained at 290 °C, suggested a possibility of formation of gaseous by-products, probably, emanating from cracking of volatile substances, under a long residence time of 4 h. These by-products may

include 1-tetracosanol, decylcyclopentane, 4-methyl tetradecane, tridecane, dodecane, undecane, nonane, octane and hexadecane, as suggested by Fu et al. (2010).

Similarly, the highest selectivity of pentadecane (24.4%) was obtained at 290°C and the lowest of 6.8% at 330°C. For heptadecane, the highest and lowest selectivities of 3.3% and 2.3% were obtained at 290°C and 330°C, respectively. This shows that at 290 °C, the highest selectivity favoured the production of pentadecane, which is the main product of the decarboxylation of palmitic acid, as expected. The selectivity was found to decrease with increase in temperature and this corroborates the findings by Fu et al. (2010). In principle, this is presumably due to the inverse proportionality between selectivity and conversion which is projected to increase with temperature (Fu et al., 2011b, Maki-Arvela et al., 2011).

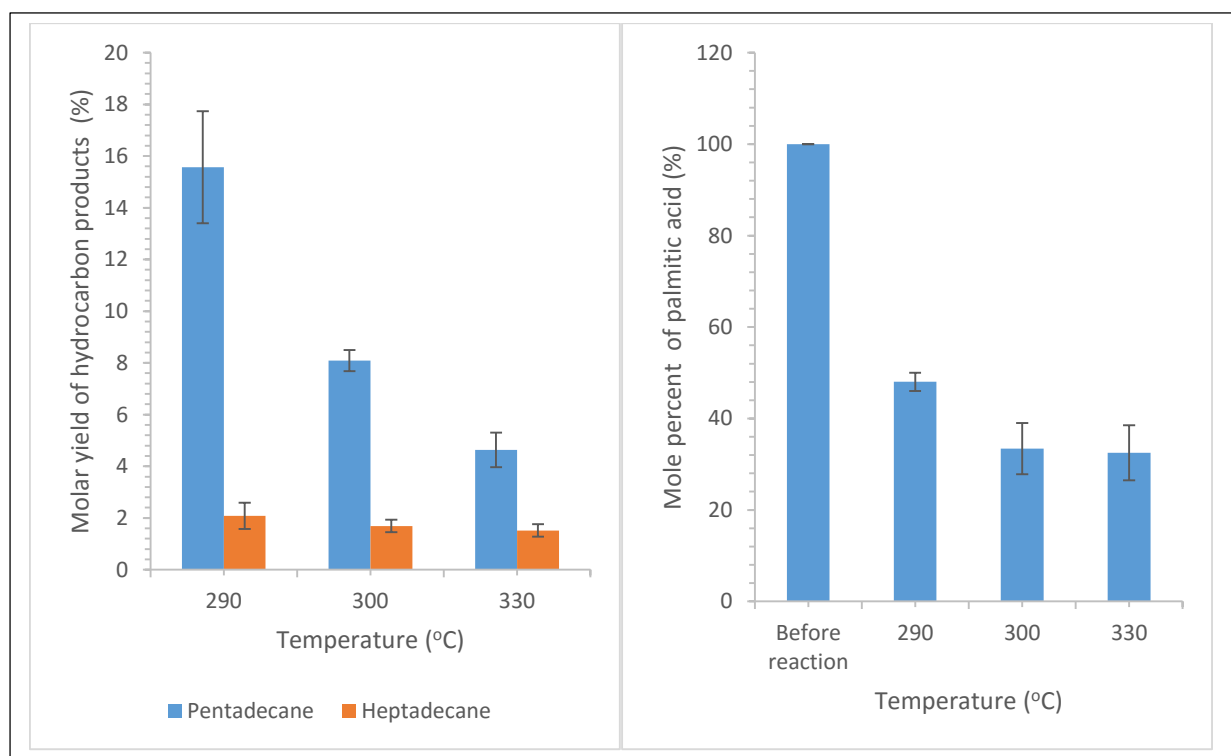


Figure 5-1: effect of temperature on the yield of hydrocarbon products and palmitic acid consumption at constant time of 4h using palladium on activated carbon

In conclusion, considering the yield and selectivity of the hydrocarbon products, the optimum conditions for the production of pentadecane and heptadecane were 290°C, 4h, 20 mg of Pd/C

and water loading of 15 mL, Figures (5-1 and 5-2). The conversion of palmitic acid was higher at 300°C and 330°C compared to 290°C indicating the possibility of the formation of by-products at these temperatures.

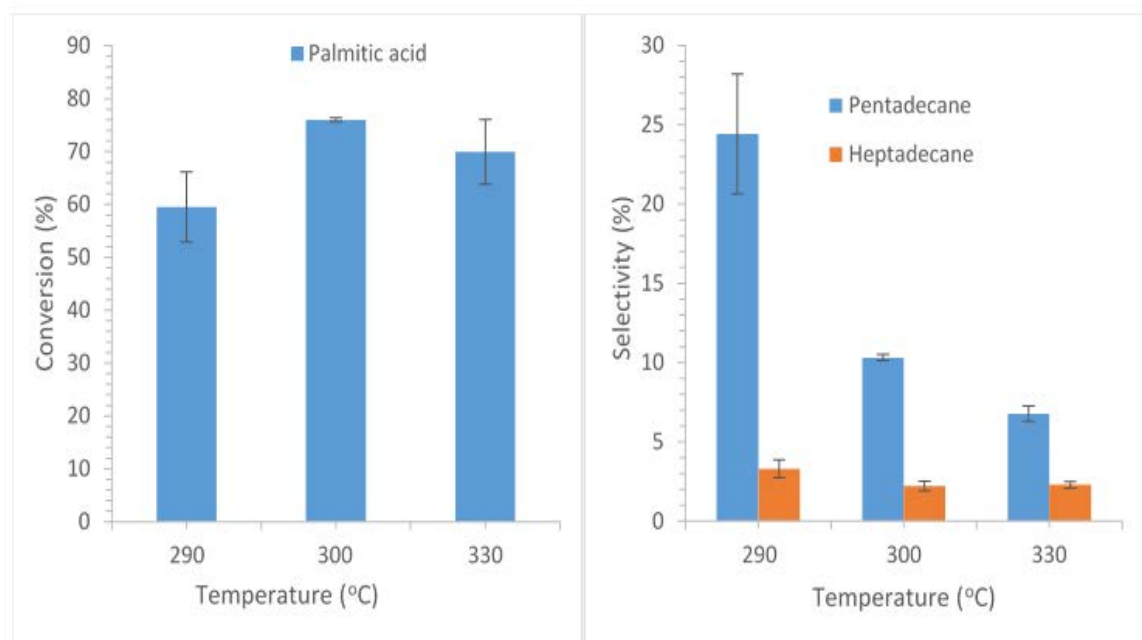


Figure 5-2: effect of temperature on conversion of palmitic acid and selectivity of hydrocarbon products (pentadecane and heptadecane) from hydrothermal decarboxylation at constant residence time of 4h using palladium on activated carbon catalyst. Where selectivity (%) = (number of moles of products recovered / (initial number of moles of reactant – number of moles of product recovered x 100)

5.2.1.1.2 Effect of residence time on hydrothermal decarboxylation of palmitic acid using palladium on activated carbon (Pd/C) catalyst at 290°C

Following the optimum temperature of 290°C obtained in Section 5.2.1.1.1, further catalytic hydrothermal decarboxylations of palmitic acid were carried out at varied residence times (0.5, 1, 1.5 and 3 h) in order to determine the optimum conditions that favour higher yield and selectivity of hydrocarbon products. The experiments were conducted according to the method described in Section 2.7.4.2 using 100 mg of palmitic acid, 20 mg of Pd/C catalyst and 15 mL of water. The results of the effect of residence time on the yield of the hydrocarbon products:

pentadecane and heptadecane are presented in Figure 5-3. The maximum and minimum molar yields of pentadecane were 35.4 % and 5.9 % obtained at 1.5 h and 0.5 h, respectively. That of heptadecane were 4.5 % and 2.0 % obtained at 1 h and 0.5 h, respectively. These results when compared to those presented in Section 5.2.1.1.1 show that the molar yield increased from 15.6 % at 4 h and 35.4 %, when the time of decarboxylation was reduced to 1.5 h at 290 °C, respectively (Figures 5-1 and 5-3). With this increment in molar yield, it appeared that residence time of 4 h was in excess to that which was required to ensure maximum yield of pentadecane. The decrease in molar yield could be attributed to gasification, catalyst deactivation and cracking of volatile substances, although, 290 °C is lower than the supercritical temperature (Fu et al., 2010, Kruse and Gawlik, 2003). It was shown in Figure (5-3) that the molar yield of pentadecane after 3 h of decarboxylation was 30.0 %, contrary to that which was obtained by Fu et al. (2010) at the same residence time. They presented 63 % of pentadecane, at 370 °C for 3 h decarboxylation, using 20 mg of 5% Pd/C and 0.167 mL of water. The lower molar yield might be due to difference in temperature and volume of water used. Fu et al. (2010) had used 0.167 mL whereas, 15 mL was employed here and this presumably will affect the effect of temperature on the decarboxylation reaction.

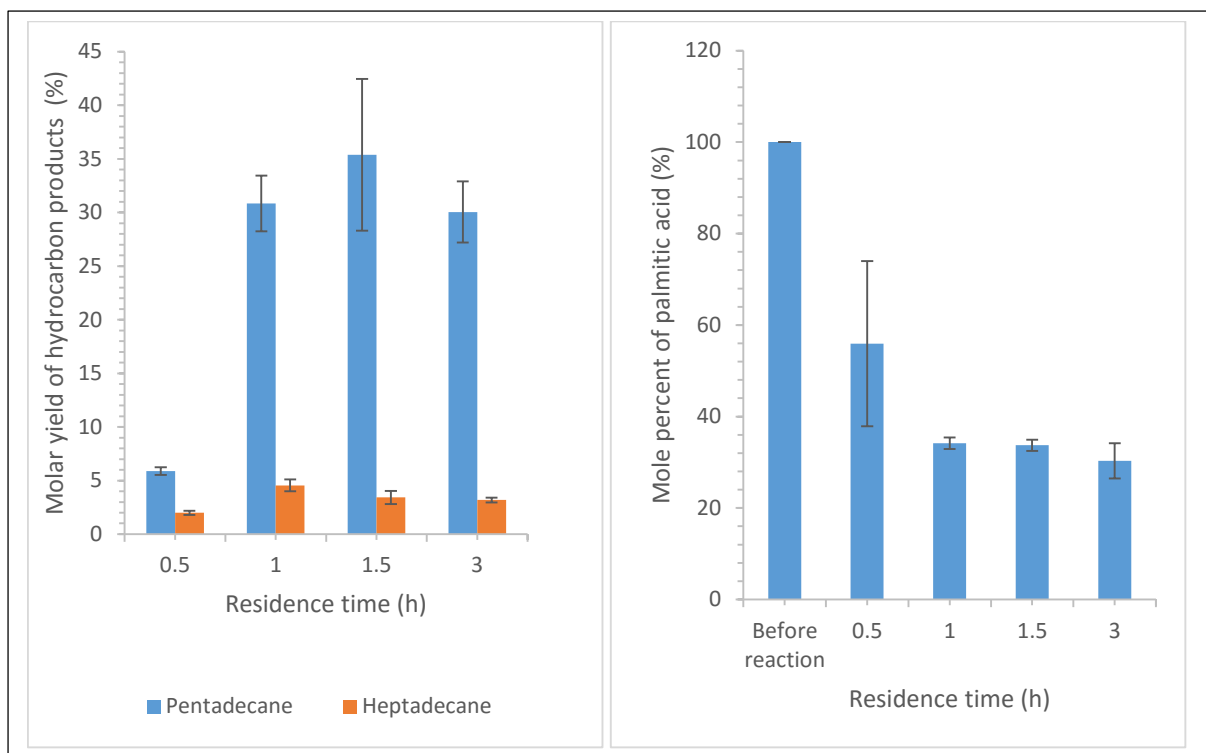


Figure 5-3: effect of residence time on yield of hydrothermal decarboxylation of palmitic acid using Pd/C at 290°C

The mole percent of palmitic acid was assumed to be 100 % before the reaction and this decreased to 30.3 %, after 3 h, as the decarboxylation progressed. The results of the conversion of palmitic acid and selectivity of the hydrocarbon products with respect to residence time are depicted in Figure 5-4. The maximum and minimum conversions of palmitic acid were 71.7% and 66.3 % obtained at 1 h and 1.5 h, respectively. Those of the selectivities of pentadecane were 54.6 % and 8.1 % obtained at 1.5 h and 0.5 h, respectively. Similarly, the maximum and minimum selectivities of heptadecane were 4.9 % and 2.9 % obtained at 1.5 h and 0.5 h, respectively. Thus, the optimum selectivity was obtained at 1.5 h, favouring the production of pentadecane which is the main hydrocarbon product of the decarboxylation of palmitic acid, as evidenced by 35.4 % molar yield, against 3.4 % of heptadecane, obtained at this condition.

The yields of the hydrocarbon products: pentadecane and heptadecane show that 1h was the optimum residence time required to carry out the hydrothermal decarboxylation of palmitic acid at a temperature of 290°C, Pd/C catalyst loading of 20 mg and water loading of 15 mL (Figure 5-3). The selectivity results suggest that longer residence time of at least 1.5 h favoured the production of pentadecane and a maximum of 1.5 h was required to obtain more of heptadecane, see Figure 5-4. Similarly, the maximum conversion of 71.7 % at 1 h favoured the production of more heptadecane than pentadecane. The results, thus obtained have illustrated that palladium on activated carbon can be used to catalyse the hydrothermal decarboxylation of palmitic acid leading to the production of pentadecane, as the primary hydrocarbon, without the addition of hydrogen. The optimum conditions that resulted to the highest molar yield (35.4 %) of pentadecane were temperature (290°C), residence time (1.5 h), catalyst loading (20 mg) and water loading (15 mL).

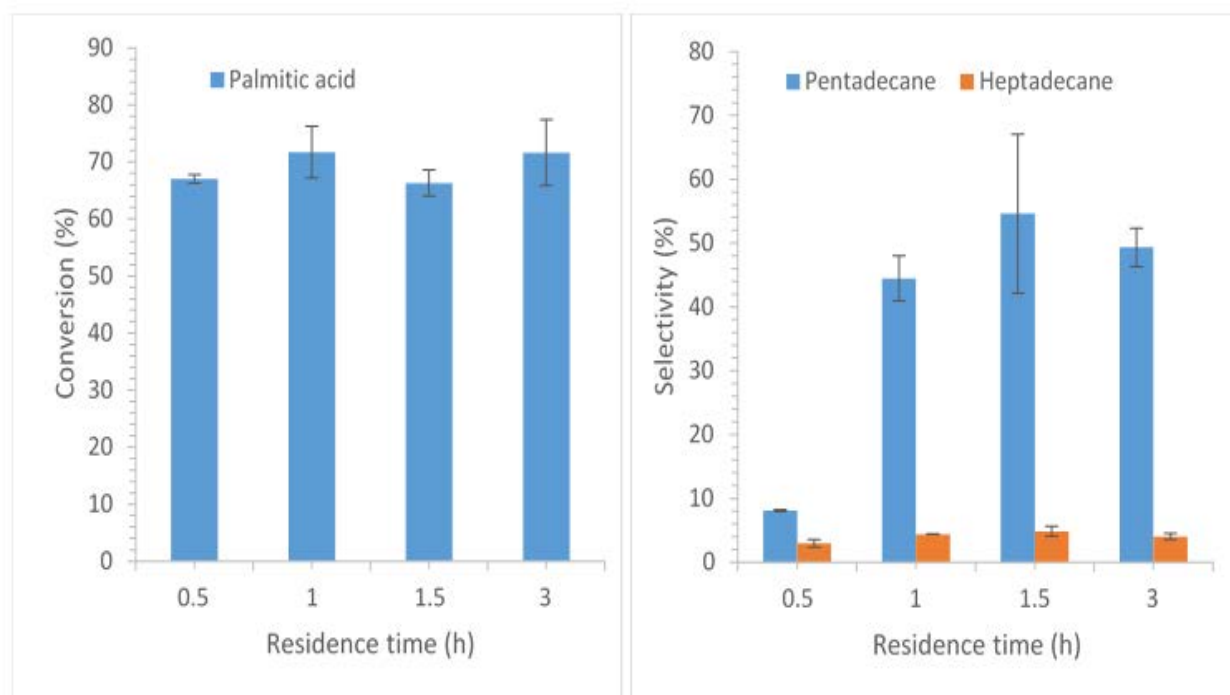
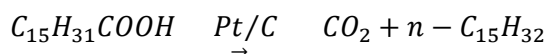


Figure 5-4: effect of residence time on conversion of palmitic acid and selectivity of hydrocarbon products (pentadecane and heptadecane) from hydrothermal decarboxylation at a constant temperature of 290°C using palladium on activated carbon catalyst. Where selectivity (%) = (number of moles of products recovered / (initial number of moles of reactant – number of moles of product recovered x 100)

5.2.1.2 Hydrothermal decarboxylation of palmitic acid using platinum on activated carbon (Pt/C) catalyst at a residence time of 4h

This was carried out in order to evaluate the efficacy of Pt/C catalyst in the hydrothermal decarboxylation of palmitic acid to hydrocarbon products without the addition of hydrogen. The effects of temperatures (290, 300 and 330°C) and residence times (0.5, 1, 1.5 and 3 h) were investigated using 100 mg of palmitic acid, 20 mg of Pt/C catalyst and 15 mL of water. The GC chromatograms with the yields and selectivities of the hydrocarbon products obtained from the conversion of palmitic acid using Pt/C catalyst are presented. The reaction scheme is presented below for the selective conversion of palmitic acid ($C_{15}H_{31}COOH$) to pentadecane ($n - C_{15}H_{32}$) using platinum on activated carbon (Pt/C) catalyst. Heptadecane was identified as a secondary product, while other products suspected were those reported by Fu et al. (2010)

which include 1-tetracosanol, decylcyclopentane, 4-methyl tetradecane, tridecane, dodecane, undecane, nonane, octane and hexadecane.



Scheme 5-2: chemical reaction illustrating the conversion of palmitic acid in the presence of Pt/C to pentadecane

5.2.1.2.1 Effect of temperature on hydrothermal decarboxylation of palmitic acid using platinum on activated carbon (Pt/C) catalyst at a residence time of 4h

This was evaluated in order to determine the optimum temperature that favours the hydrothermal decarboxylation of palmitic acid at a constant residence time of 4 h. The experiments were conducted, according to the method described in Section 2.7.4, at the temperatures of 290, 300 and 330°C. The molar yields of the hydrocarbon products: pentadecane and heptadecane obtained with respect to temperature are presented in Figure 5-5. The maximum and minimum molar yields of pentadecane were 20.0 % and 17.8 %, obtained at 300 °C and 330 °C, respectively. Those of heptadecane were 5.3 % and 3.1 %, obtained at 290 °C and 330 °C. For palmitic acid, the mole percent was taken to be 100 %, before the reaction and this decreased to 25.0 %, after 4 h, see Figure (5-5). Thus, the results show that temperature had a slight impact on the molar yield of hydrocarbons produced, under the experimental conditions investigated, using platinum on carbon catalyst (Figure 5-5).

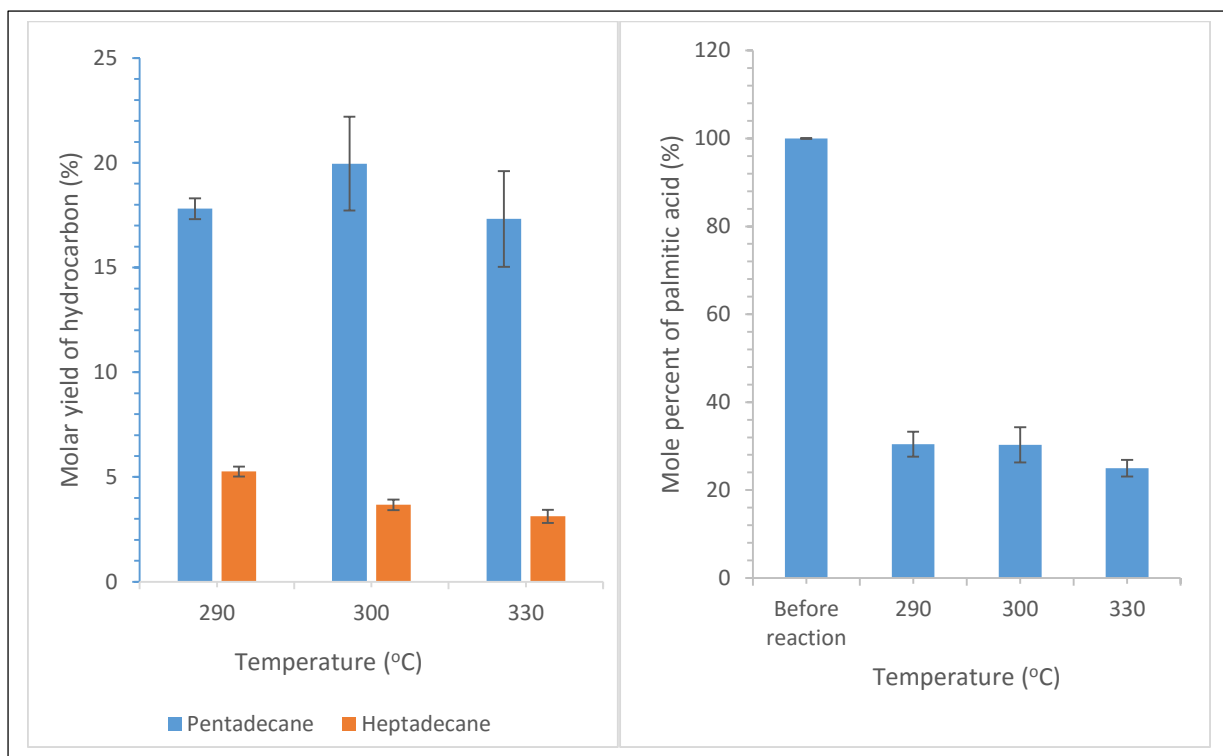


Figure 5-5: effect of temperature on yield of hydrothermal decarboxylation of palmitic acid using Pt/C at constant residence time of 4h

The results of conversion of palmitic acid and the selectivities of the hydrocarbon products are presented in Figure 5-6. The conversion of palmitic acid increased slightly from 63.4 % to 64.2 % at 290 °C and 300 °C, respectively, before, decreasing to 36.5 % at 330 °C. On the other hand, the selectivity of pentadecane, increased from 29.2 % to 46.8 %, as the temperature increased from 290 °C to 330 °C. While the maximum and minimum selectivities of heptadecane were 8.1 % and 5.4 %, obtained at 290°C and 300 °C, respectively. Thus, selectivity of pentadecane increased as temperature increased, with the highest (46.8 %) obtained at 330 °C and its molar yield was found to increase from 17.8 % at 290 °C to 20.0 % at 300 °C and then decreased to 17.3 % at 330 °C. This indicates that 300 °C was the optimum condition and was supported by the highest conversion (64 %) of palmitic acid obtained at this temperature. The highest values of molar yield and selectivity of pentadecane compared to heptadecane, thus, supports it as the main hydrocarbon product of deoxygenation of palmitic acid (Figures 5-5 and 5-6).

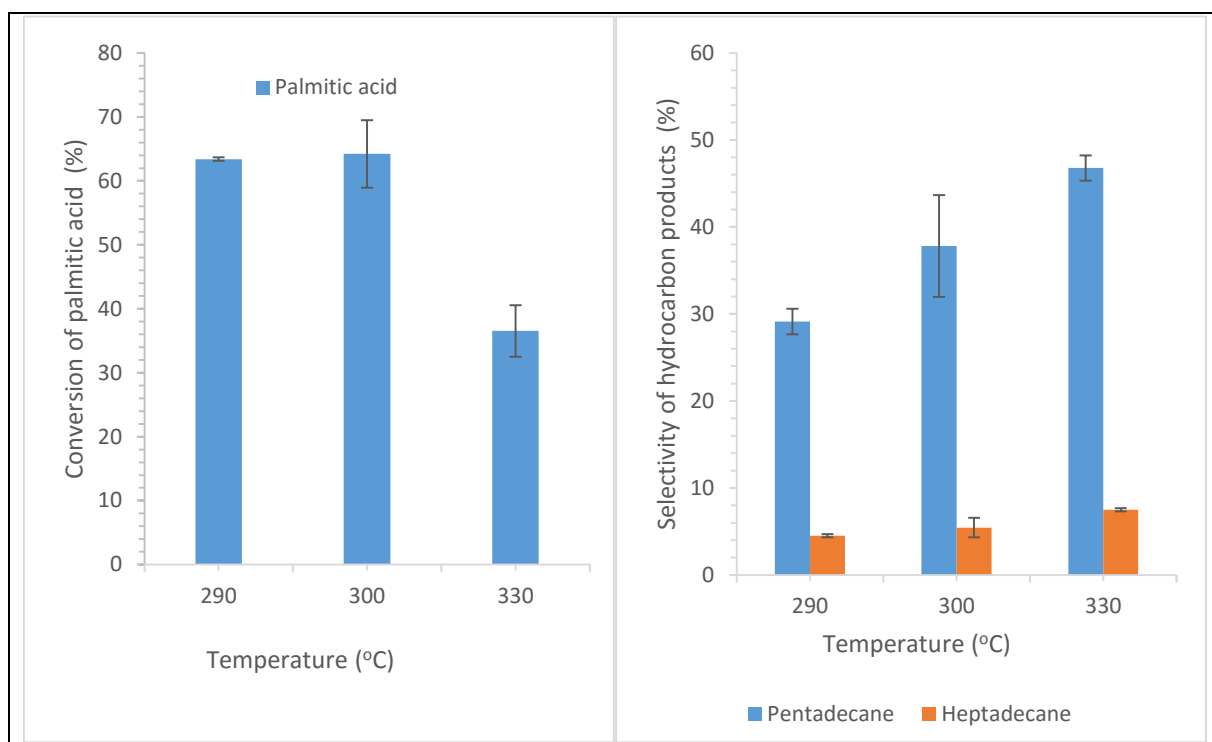


Figure 5-6: effect of temperature on conversion of palmitic acid and selectivity of hydrocarbon products from hydrothermal decarboxylation at constant residence time of 4 h using platinum on activated carbon catalyst. Where selectivity (%) = (number of moles of products recovered / (initial number of moles of reactant – number of moles of product recovered x 100)

The results presented above, suggest that after 4 h, using 100 mg of palmitic acid, 20 mg of Pt/C catalyst and 15 mL of water, that temperature had some influence on the molar yield of pentadecane production. Although, the optimum temperature was 300 °C, but, since the difference was not appreciable, for economic reasons, 290 °C could be used for further investigation on the influence of residence time, in order to maximize the hydrocarbon production.

5.2.1.2.2 Effect of residence time on performance of hydrothermal decarboxylation of palmitic acid using platinum on activated carbon (Pt/C) catalyst at 290°C

In order to maximize the molar yield of the hydrocarbon products, further investigations were carried out at 290 °C to determine the optimum residence time required in the hydrothermal

decarboxylation of palmitic acid using Pt/C catalyst. The experiments were conducted according to the procedure presented in Section 2.7.4.2, using 20 mg of Pt/C catalyst, 100 mg of palmitic acid and 15 mL of water. The molar yield and mole percent of hydrocarbon products and palmitic acid are presented in Figure 5-7. The yield of pentadecane increased from 24.8 % at 0.5 h to the optimum of 55.3 % at 1.5 h and then descended to 27.6 % at 3 h. Similarly, heptadecane followed the same trend as the pentadecane. It increased from 1.7 % at 0.5 h to the maximum value of 4.4 % at 1.5 h, before declining to 3.1 % at 3 h. While the mole percent of palmitic acid, decreased from 100 % before the reaction to 21.0 %, after 3 h of the hydrothermal decarboxylation.

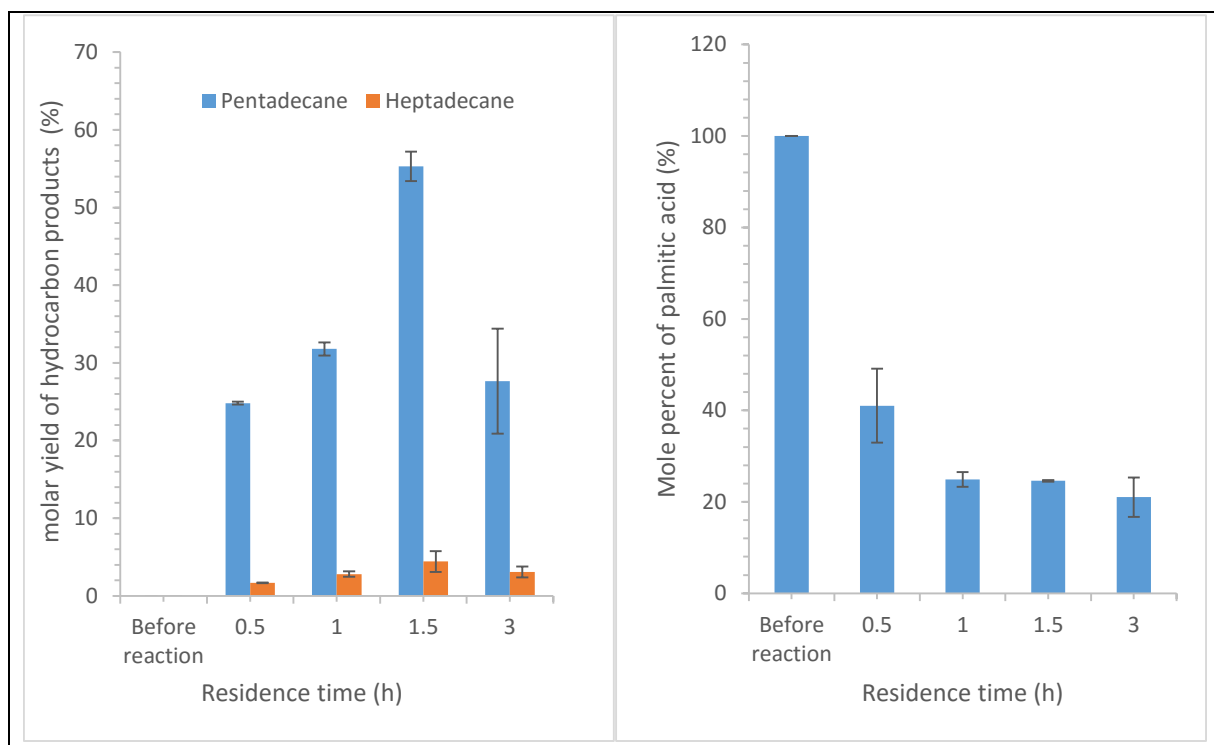


Figure 5-7: effect of residence time on yield of hydrothermal decarboxylation of palmitic acid using Pt/C at constant temperature of 290°C

The results of the conversion of palmitic acid and the selectivities of the hydrocarbon products are presented in Figure 5-8. The lowest conversion was 48.7 % and the highest was 79.1 % obtained at 0.5 h and 3 h, respectively. The maximum and minimum selectivities of

pentadecane were 89.1 % and 36.2 %, obtained at 1.5 h and 3 h, respectively. Those of heptadecane were 7.3 % and 3.1 %, obtained at 1.5 h and 0.5 h, respectively.

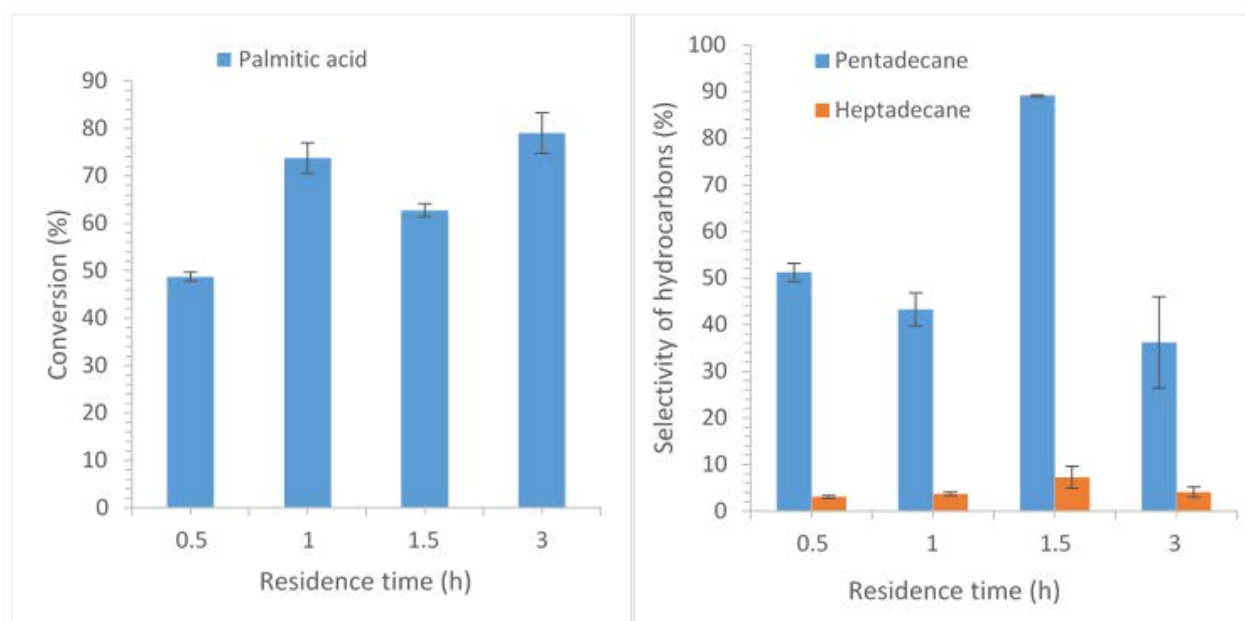


Figure 5-8: effect of residence time on conversion of palmitic acid and selectivity of hydrocarbon products from hydrothermal decarboxylation at constant temperature of 290°C using platinum on activated carbon catalyst. Where selectivity (%) = (number of moles of products recovered / (initial number of moles of reactant – number of moles of product recovered x 100)

Considering the results presented above, residence time at constant temperature of 290 °C influenced the yield of hydrocarbons, favouring pentadecane with 55.3 % and selectivity of 89.1 % at 1.5 h. At these conditions, the molar yield of pentadecane was 55.3 % compared to 20.0 % obtained at 300 °C in a decarboxylation of 4 h (Figures 5-7 and 5-8). Thus, resulting to an increase of 35.3 % molar yield (Section 5.2.1.2.1). The maximum conversion that was obtained was 79.1 %, after 3 h at 290 °C. Since the molar yield of pentadecane obtained at these conditions was less than that from a lower residence time of 1.5 h and conversion of 62.7 %, there is possibility of formation of by-products such as 1-tetracosanol, decylcyclopentane, 4-methyl tetradecane, tridecane, dodecane, undecane, nonane, octane and hexadecane (Fu et al., 2010).

5.2.1.2.3 Effect of catalyst on the hydrothermal decarboxylation of palmitic acid

1. Effect of catalyst and its type on yield and selectivity of hydrocarbons, and conversion of palmitic acid using hydrothermal decarboxylation at constant temperature of 290 °C and residence time of 4 h

This was evaluated in order to determine whether the use of catalysts was important and which of them had greater influence on the deoxygenation of palmitic acid, under the same operating conditions. To achieve this, experiments were carried out without the use of catalyst and in the presence of catalysts: Pd/C and Pt/C, at constant temperature (290 °C) for 4 h, using 100 mg of palmitic acid, 20 mg of catalyst and 15 mL of water, according to the method described in Section 2.7.4.1. The results obtained, expressed in terms of molar yields and selectivities of pentadecane and heptadecane, and conversion of palmitic acid are presented in Figure 5-9. The molar yields of pentadecane (17.8 %) and heptadecane (5.3 %) were highest when the reaction was catalysed using Pt/C and were lowest without catalyst given 4.5 % and 1.2 %, respectively. Similarly, the selectivity was highest using Pt/C with 29.2 % and lowest without catalyst given 10.7 %, favouring pentadecane, respectively. The maximum conversion of palmitic acid (63.4 %) was obtained using Pt/C and minimum (25.1 %) without catalyst. Considering these results, it can be inferred that catalyst had a great impact on the performance of hydrothermal decarboxylation of palmitic acid and that Pt/C showed a greater catalytic effect compared to Pd/C. This was evidenced in the higher molar yields, selectivities and conversions of 100 mg of palmitic acid to the main hydrocarbon product which is pentadecane, at constant temperature of 290 °C, for 4 h decarboxylation, obtained using 20 mg of 5% Pt/C than 20 mg of 5% Pd/C.

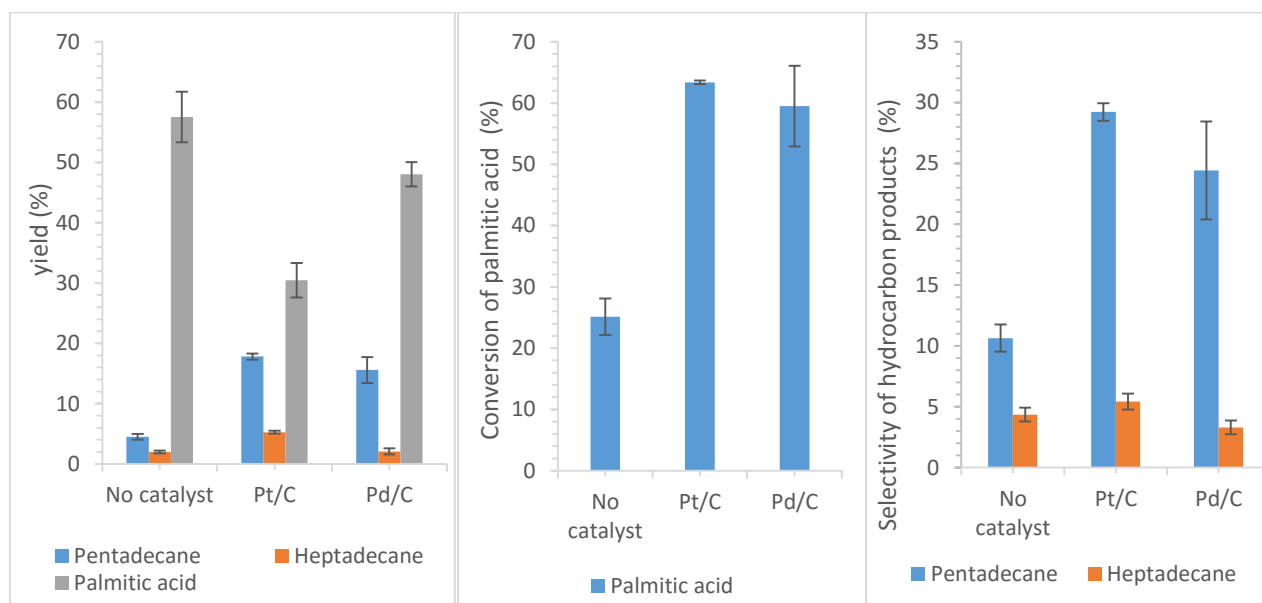


Figure 5-9: effect of catalyst on yield and selectivity of hydrocarbons (pentadecane and heptadecane) and conversion of palmitic acid using hydrothermal decarboxylation at 290°C for 4 h. Where selectivity (%) = (number of moles of products recovered / (initial number of moles of reactant – number of moles of product recovered x 100). For “No catalyst”, the reaction was still carried out 290 °C.

Higher catalytic effects using Pt/C than Pd/C was also recorded under varied residence times (0.5, 1 and 1.5 h), resulting to an optimum molar yield of pentadecane of 55.3 %, at constant temperature of 290 °C, although, at 3 h, Pd/C gave a slight greater molar yield, this might be due to experimental error. The greater catalytic effect observed using Pt/C at 290 °C corroborates that presented by Fu et al. (2010), in which they demonstrated that Pt/C showed a higher catalytic effect in hydrothermal decarboxylation of palmitic acid, as seen by 63 % and 76 % molar yield of pentadecane obtained using 20 mg of 5 % Pd/C for 3 h and 15 mg of 5 % Pt/C for 1 h, respectively, at 370 °C. The reason for this higher efficacy might be attributed to the suspected loss in catalyst activity experienced using Pd/C in decarboxylating palmitic acid in water than with Pt/C (Fu et al., 2010). This might be connected to the reduction in the surface areas of the catalyst supports, micropore volume and metal dispersion encountered during the hydrothermal process, as Fu et al. (2010), has said that there could be loss in catalyst activity if the reaction conditions causes loss in surface area or uncovered metal sites.

2. Effect of catalyst type on hydrocarbon yield from the hydrothermal decarboxylation of palmitic acid at 290 °C and varied residence time

This was conducted to assess the impact of catalyst type: Pd/C and Pt/C on the yield of hydrocarbons at a constant temperature of 290 °C and varied residence times (0.5, 1, 1.5 and 3 h), using hydrothermal decarboxylation of palmitic acid. The respective results of molar yields of pentadecane and heptadecane, obtained using Pd/C and Pt/C have been presented in Sections 5.2.1.1.2 and 5.2.1.2.2, but combined in Figure 5-10. From this Figure, the maximum and minimum molar yields of pentadecane were 35.4 % and 5.9 %, obtained after 1.5 h and 0.5 h, using Pd/C, respectively, and those of heptadecane were 4.5 % and 2.0 %, derived after 1 h and 0.5 h decarboxylations, respectively. While the maximum and minimum molar yields of pentadecane, obtained using Pt/C were 55.3 % and 31.8 %, and those of heptadecane were 4.4 % and 1.7 %, after 1.5 h and 0.5 h decarboxylation, respectively. The results thus, show that Pt/C had a better impact on the reaction, resulting to the highest molar yield of 55.3 % of pentadecane.

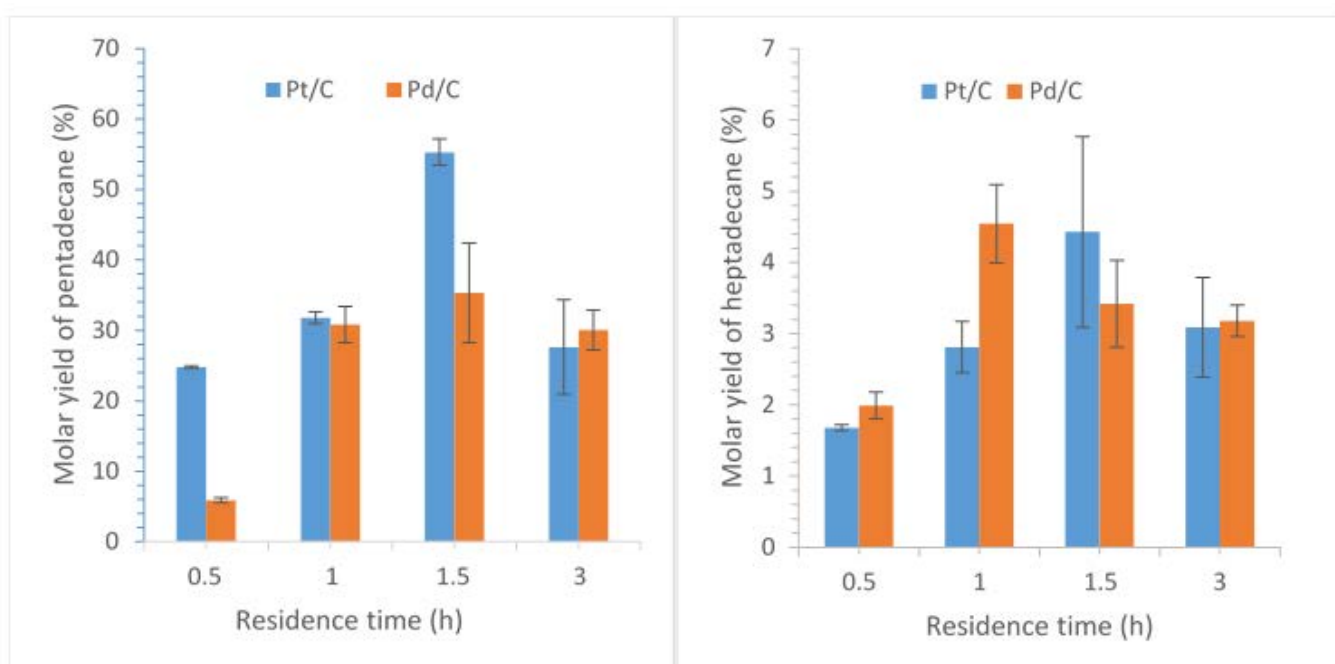
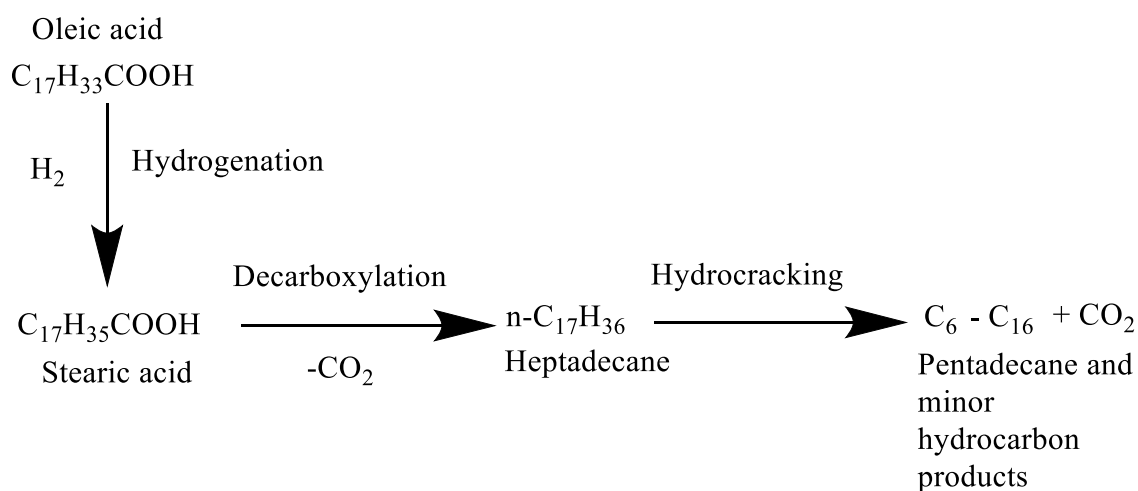


Figure 5-10: effect of catalyst type on yield of hydrocarbon products from the hydrothermal decarboxylation of palmitic acid at 290°C and varied residence time

5.2.2 Hydrothermal decarboxylation of oleic acid

As the lipid extract from the activated sludge contains some amount of oleic acid, it was necessary to carry out hydrothermal decarboxylation of a commercial oleic acid in order to aid understanding of the reaction involving the lipid extract. The efficacies of the Pd/C and Pt/C in converting oleic acid to hydrocarbons, with heptadecane as the primary hydrocarbon product, were evaluated at varied temperatures and residence time. This was done in order to find out the most suitable catalyst for the hydrothermal decarboxylation of oleic acid. The suspected reaction mechanism of the process is presented in Scheme 5-3. The prevalent reaction pathway was the formation of stearic acid through the hydrogenation of the double bond in oleic acid, using hydrogen that might be produced from a reaction between water and carbon support or from water shift reaction (Fu et al., 2011b). The deoxygenation of the –COOH group in stearic acid to heptadecane and CO₂ was by decarboxylation reaction in the presence of Pd/C or Pt/C. Due to possibility of hydrocracking of heptadecane at high temperature, C₆-C₁₆ was expected (Ahmadi et al., 2014).



Scheme 5-3: reaction mechanism illustrating the conversion of oleic acid to hydrocarbons using Pd/C and Pt/C

5.2.2.1 Hydrothermal decarboxylation of oleic acid using palladium on activated carbon (Pd/C) catalyst

This was conducted to investigate the potential of Pd/C catalyst in influencing the rate of hydrocarbon production from the hydrothermal decarboxylation of oleic acid under the operating conditions considered. Here, the effects of temperature and residence time on hydrothermal decarboxylation of oleic acid under the influence of palladium on activated as catalyst are also discussed. Much emphasis was made on heptadecane than pentadecane, since they were the target hydrocarbon products and had higher yields. Generally, stearic acid was observed to be the main product of decarboxylation, as it was higher than other products at all the operating conditions considered. This is not good, as the primary aim was not produce heptadecane.

5.2.2.1.1 Effect of temperature on hydrothermal decarboxylation of oleic acid using palladium on activated carbon (Pd/C) catalyst

This was done in order to study the impact of temperatures (290, 300 and 330°C) on the hydrothermal decarboxylation of oleic acid using Pd/C catalyst. The experimental procedures are as shown in Section 2.7.4 and were carried out using 100 mg of oleic acid, 20 mg of Pd/C catalyst, 15 mL of water, at varied temperatures of 290, 300 and 330°C. The molar yields of the products recovered at the end of the reaction are presented in Figure 5-11. The maximum and minimum yields of pentadecane were 3.0% and 2.5 %, and those of heptadecane were 16.6 % and 10.5%, obtained at 330 °C and 290 °C, respectively. For the free fatty acids, the highest and lowest molar yields of stearic acid were 44.5 % and 38.0 % obtained at 290 °C and 330°C, respectively and those of palmitic acid were 13.6 % and 10.5 %, obtained at 300 °C and 290°C. As expected, oleic acid was 100 % before the reaction and decreased, as the reaction progressed to 11.9 %, after 4 h at 330°C.

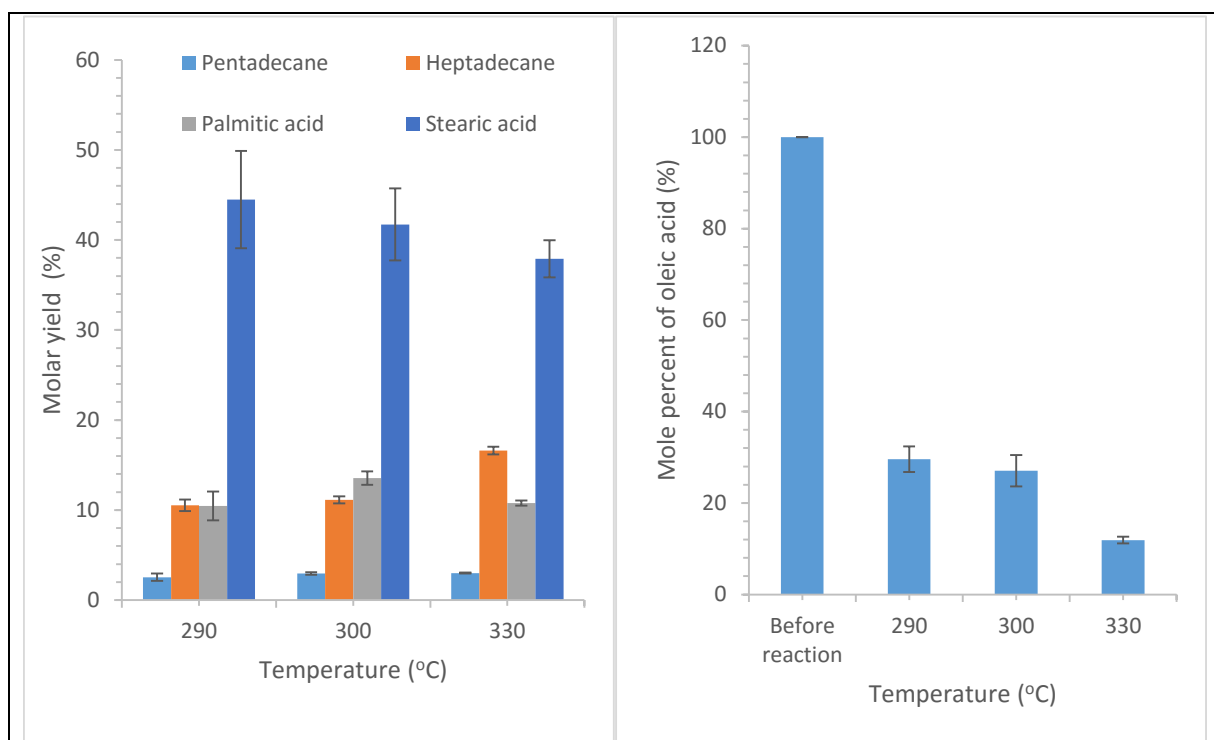


Figure 5-11: effect of temperature on the products of hydrothermal decarboxylation of oleic acid using palladium on activated carbon at a residence time of 4h

The results of the conversion of oleic acid and selectivity of the hydrocarbon products are presented in Figure 5-12. The maximum conversion was 88.1% and the minimum was 73.3 % obtained at 330°C and 290 °C, respectively. For the selectivity of the hydrocarbons products, 4.1 % and 3.2 % were obtained at 300 °C and 290 °C, representing the highest and lowest values of pentadecane, respectively. Similarly, the maximum selectivity of heptadecane was 16.9 % and minimum was 14.7 %, obtained at 330°C and 290 °C, respectively.

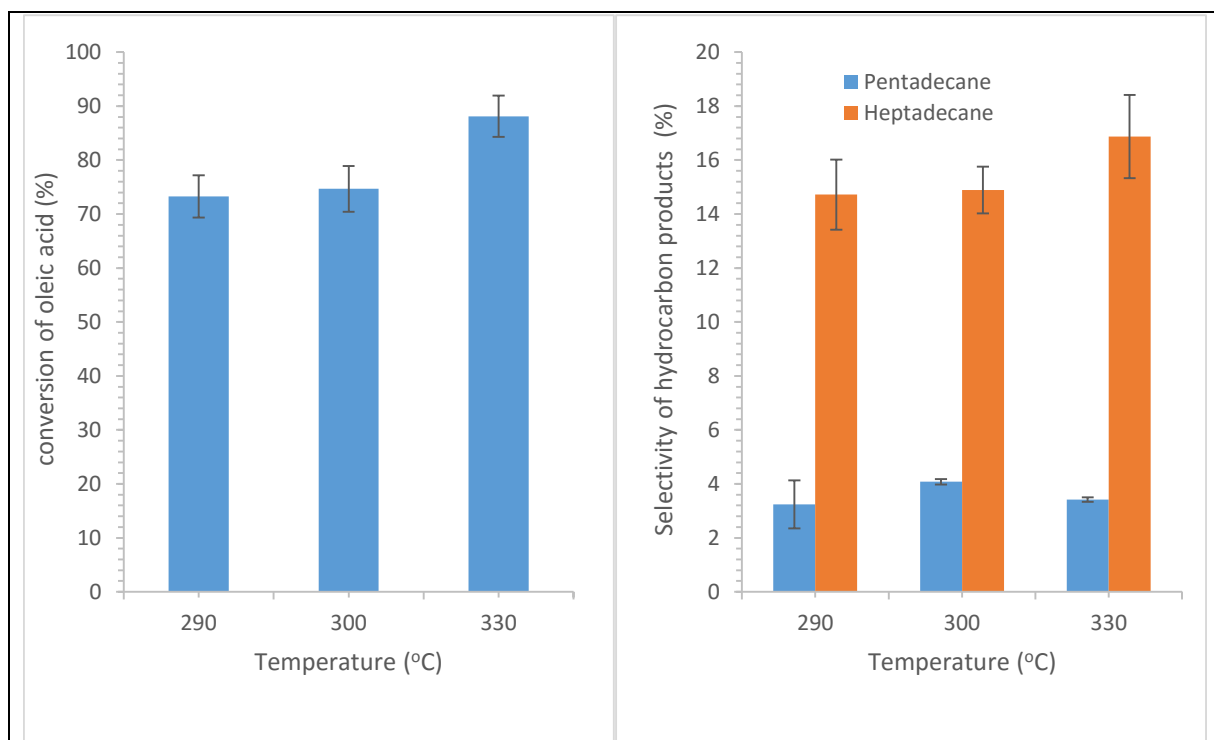


Figure 5-12: effect of temperature on conversion of oleic acid and selectivity of hydrocarbon products from hydrothermal decarboxylation at constant residence time of 4 h using palladium on activated carbon catalyst. Where selectivity (%) = (number of moles of products recovered / (initial number of moles of reactant – number of moles of product recovered x 100)

Considering the results presented above, the effect of temperature shows that high temperature of 330°C is required in order to get a maximum yield of hydrocarbon products (pentadecane and heptadecane). The highest selectivity obtained at 330 °C favoured the production of heptadecane, as expected, other hydrocarbon product identified was pentadecane, and free fatty acids recovered at the end of the reactions were oleic acid, palmitic acid and stearic acid. The maximum molar yield of heptadecane (16.6 %) and conversion of oleic acid (88.1 %) were also found at this optimum temperature (330 °C). The molar yield of stearic acid at all the three temperatures investigated was found to be greater than that of heptadecane, probably due to suspected hydrogenation reaction (Fu et al., 2011a). Hydrogenation reaction might have occurred with hydrogen produced from the possible reaction between water and carbon catalyst

or from water gas shift reaction (Fu et al., 2011a). The decrease in the molar yield of stearic acid from 44.5 % to 37.9 % and increase in that of heptadecane from 10.5 % to 16.6 %, as the temperature increases, suggest that stearic acid might be an intermediate product which undergoes decarboxylation reaction to produce more heptadecane (Fu et al., 2011a). Thus, the presence of stearic acid shows that hydrogenation reaction of oleic acid preceded its decarboxylation to heptadecane. The highest conversion and selectivity obtained at 330°C favoured the production of main hydrocarbon product of decarboxylation of oleic acid which is heptadecane. Hence, hydrothermal decarboxylation of oleic acid under varied temperatures (290, 300 and 330°C) was feasible using 5 (wt. %) palladium on activated carbon.

5.2.2.1.2 Effect of residence time on the products of hydrothermal decarboxylation of oleic acid using palladium on activated carbon (Pd/C) catalyst

This was necessary in order to obtain the optimum residence time required to get a maximum yield of hydrocarbon products at constant temperature of 290 °C. The experiments were conducted according to the procedure described in Section 2.7.4 using 20 mg of Pd/C catalyst, 15 mL of water, 100 mg of oleic acid and residence times (0.5, 1, and 3 h). The results of the molar yields of the hydrocarbon products and free fatty acids are presented in Figure 5-13. The molar yield of pentadecane (4.4 %) was maximum at 1 h and 3.43 % was the minimum value obtained after 3 h. Those of heptadecane were 6.8 % and 4.8 % obtained after 3 h and 0.5 h, respectively. Similarly, the maximum and minimum molar yields of the free fatty acids: palmitic acid were 13 % and 12 %, obtained at 0.5 h and 3 h, respectively. Those of stearic acid were 42.8 % and 31.4 %, obtained at 3 h and 0.5 h. As expected there was a decline in the mole percent of oleic acid from 100 % to 29.8 %, as the reaction progressed to 3 h.

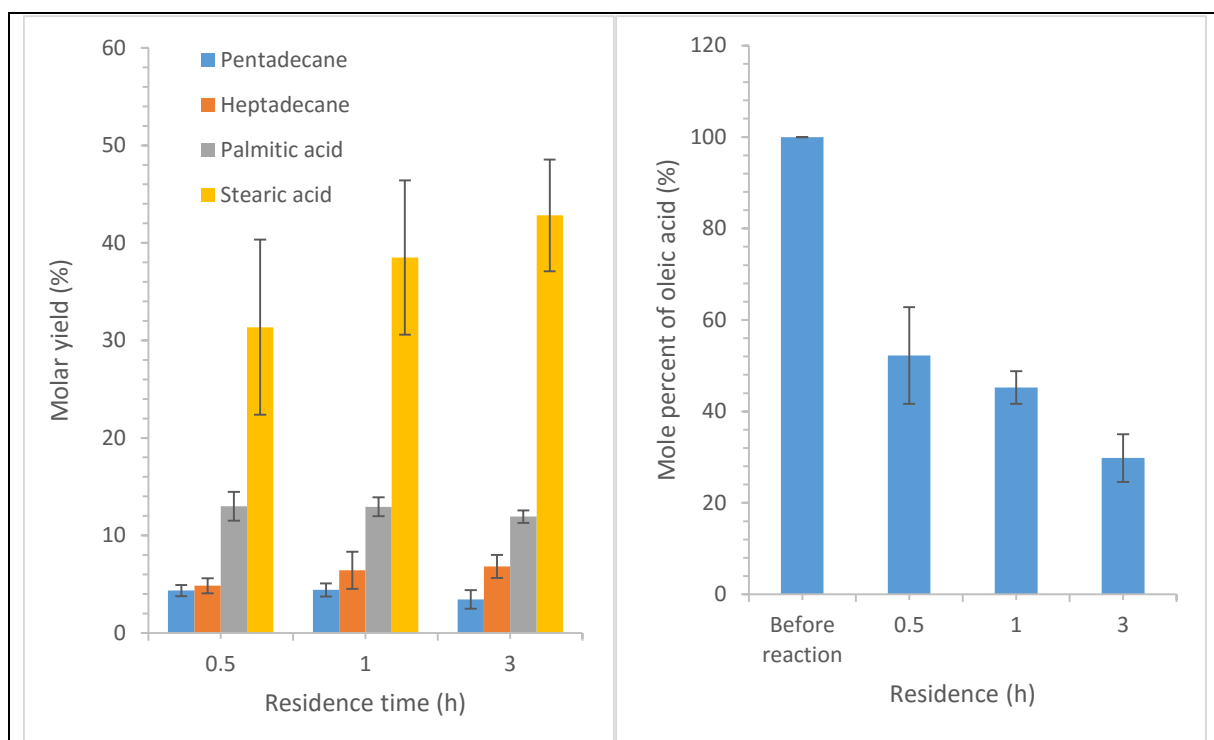


Figure 5-13: effect of residence time on molar yield of the products from hydrothermal decarboxylation of oleic acid using palladium on activated carbon at a constant temperature of 290°C

Considering the effect of residence time on the conversion of oleic acid and the selectivity of the hydrocarbon products (pentadecane and heptadecane), as presented in Figure 5-14, it is shown that the conversion (61.2 %) was highest after 3 h and lowest (47.8 %) at 0.5 h. The maximum and minimum selectivities of pentadecane were 9.2 % and 5.8 %, obtained at 1 h and 3 h, respectively. Those of the heptadecane were 13.6 % and 10.0 %, obtained at 1 h and 0.5 h, respectively. Thus, the results show that the optimum residence time for the hydrothermal decarboxylation of oleic acid to the main hydrocarbon product (heptadecane) at constant temperature of 290 °C using Pd/C catalyst is 3 h. The results also show that the optimum selectivity of heptadecane was favoured at 1 h.

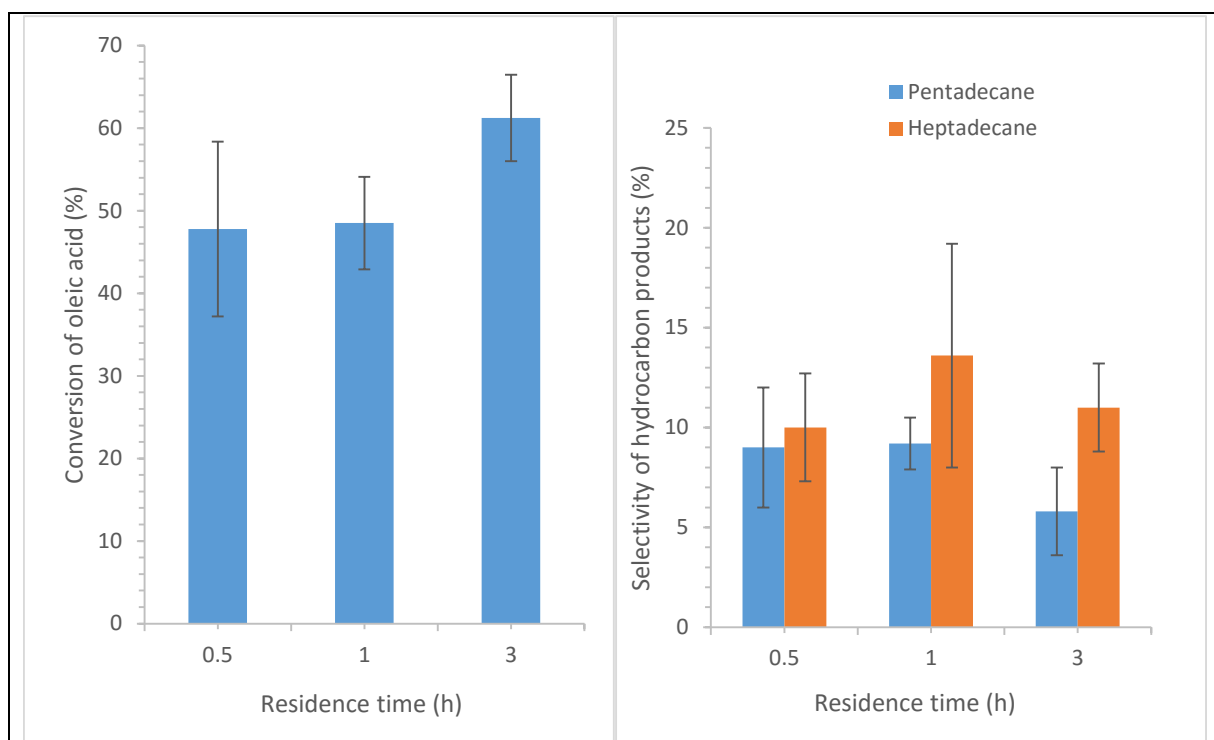


Figure 5-14: effect of residence time on conversion of oleic acid and selectivity of hydrocarbon products from hydrothermal decarboxylation at constant temperature of 290°C using palladium on activated carbon catalyst. Where selectivity (%) = (number of moles of products recovered / (initial number of moles of reactant – number of moles of product recovered x 100)

Considering, the highest selectivity of 13.6 % obtain after 1 h decarboxylation, favouring the production of heptadecane. The molar yield of heptadecane increase from 4.8 % at 0.5 h to 6.8 %, after 3 h decarboxylation. When compared, these results to the maximum molar yield of heptadecane of 16.6 % obtained at 330 °C, after 4 h, as have been presented above, it can be concluded that hydrothermal decarboxylation of oleic acid, using Pd/C performed better at a higher temperature of 330°C and longer residence time of 4 h. This could be due to low conversions of oleic acid to heptadecane obtained after 0.5 h, 1 h and 3 h decarboxylations at 290 °C, as evidenced by higher mole percent: 52.2 %, 45.2% and 29.8 %, respectively of oleic acid remaining after the reaction.

5.2.2.2 Hydrothermal decarboxylation of oleic acid using platinum on activated carbon (Pt/C) catalyst

This was done to evaluate the efficacy of Pt/C catalyst in the hydrothermal decarboxylation of oleic acid to hydrocarbon with heptadecane as the main hydrocarbon product. The yields and selectivity of the products and the conversion of oleic acid were the parameter used to assess the performance of the reaction under the influence of Pt/C as the catalyst at varied temperatures (290, 300 and 330 °C) and residence time (0.5, 1.0 and 4.0 h). The effects of operating parameters: temperature and residence time on the catalytic hydrothermal decarboxylation of oleic acid are discussed.

5.2.2.2.1 Effect of temperature on the products of hydrothermal decarboxylation of oleic acid using platinum on activated carbon (Pt/C)

This was studied in order to assess the impact of temperature on the production of hydrocarbons through the hydrothermal decarboxylation of oleic acid using Pt/C catalyst. The experiments were conducted as described in Section 2.7.4 using temperatures of (290, 300 and 330 °C), residence time of 4 h, 20 mg of Pt/C catalyst and 15 mL of water. The molar yields of the hydrocarbon products and free fatty acids recovered from the reaction are presented in Figure 5-15. The maximum and minimum molar yields of pentadecane were 3.6 % and 3.1 %, and those of heptadecane were 16.4 % and 10.7 %, obtained at 290 °C and 330°C, respectively. The highest and lowest values of palmitic acid were 17.0 % and 13.7 % and those of stearic acid were 47.4 % and 12.8%, obtained at 300 °C and 330 °C, respectively. The initial value of the oleic acid was 100 %, and this continued to decrease as the reaction progressed, resulting to mole percent of 5.8 % after 4 h at 330 °C.

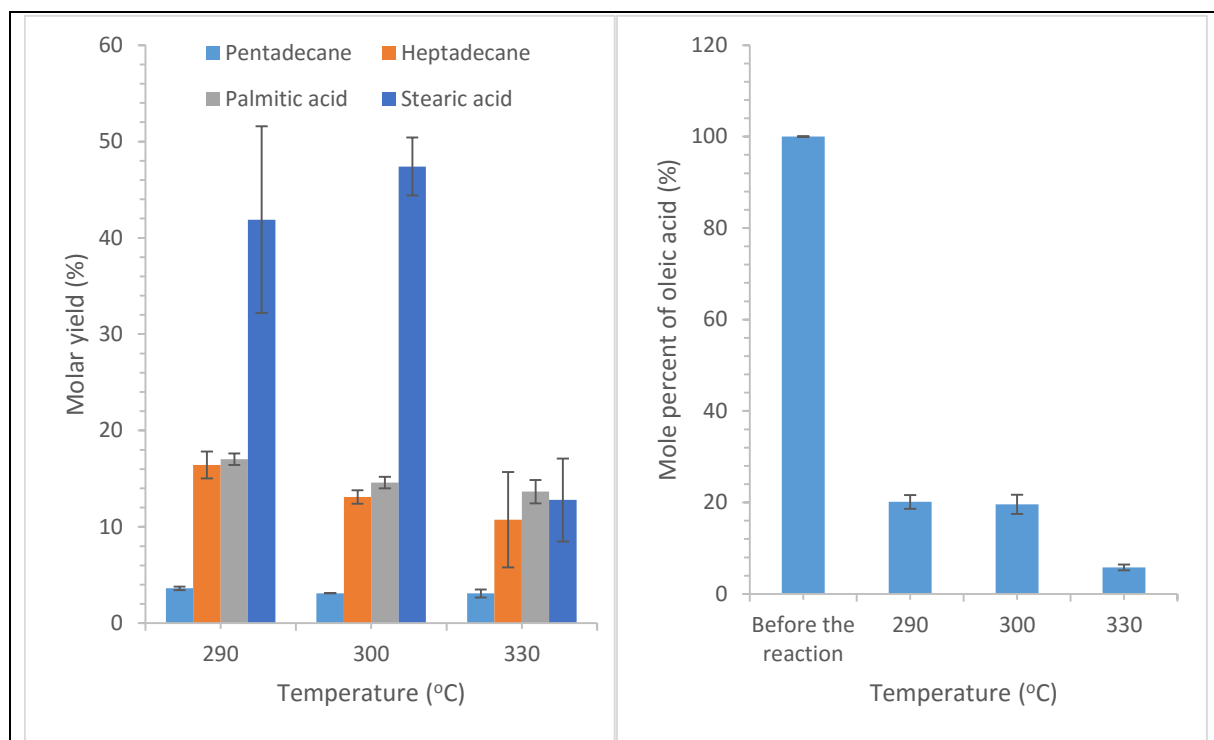


Figure 5-15: effect of temperature on the products of hydrothermal decarboxylation of oleic acid using platinum on activated carbon at a residence time of 4 h

Furthermore, the maximum and minimum conversions of oleic acid were 92 % and 79.9 %, obtained at 330 °C and 290 °C, respectively (Figure 5-16). The highest and lowest values of selectivity for pentadecane were 10.5 % and 4.0 % obtained at 330 °C and 300 °C, respectively. While those of heptadecane were 20.2 % and 3.2 %, obtained at 290 °C and 330 °C, respectively (Figure 5-16).

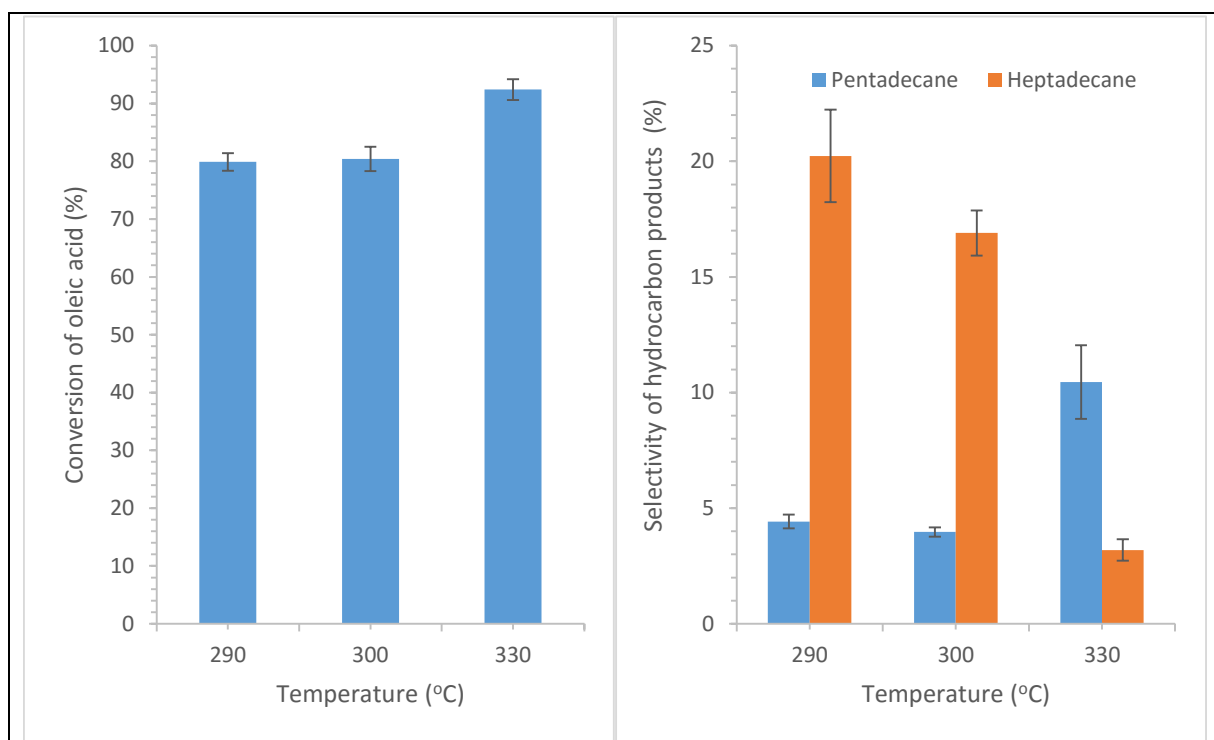


Figure 5-16: effect of temperature on conversion of oleic acid and selectivity of hydrocarbon products from hydrothermal decarboxylation at constant residence time of 4h using platinum on activated carbon catalyst. Where selectivity (%) = (number of moles of products recovered / (initial number of moles of reactant – number of moles of product recovered x 100)

In view of the results presented above, it can be concluded that temperature had some effects on the hydrothermal decarboxylation of oleic acid, although, not appreciably with pentadecane, but, with the main hydrocarbon product: heptadecane. Thus, at a constant residence time of 4 h, the optimum operating temperature was 290 °C, favouring the production of heptadecane with a molar yield of 16.42 % and selectivity of 20.2 % from oleic acid conversion of 80 %. At this temperature, the highest molar yield of stearic acid (47.4 %) was 300 °C, comparing this with 13.1 % molar yield of heptadecane, shows that the reaction path way was driven by hydrogenation of oleic acid to stearic acid. At 330 °C despite high conversion of 92.4 %, a low molar yield of 10.7 % and selectivity of 3.2 % was obtained. Upon mass balance, the percentage of pentadecane, heptadecane, palmitic acid, oleic acid and stearic acid recovered after decarboxylation at 330 °C for 4 h was 46.1 %, the remaining is attributed to other suspected

liquid products, and gaseous products which were not measured by gas chromatography in this work. The liquid products may include 2-heptanone, 2-pentanone-4-hydroxy-4-methyl, tetradecane, hexadecane, aromatic derivatives, 9-octadecanone, 1-propene-1-methoxy-2-methyl, 3-heptadecanone, 2-heptadecanone, 2(3 H)-furanone-dihydro-5-tetradecyl and heptadecenes, while the gaseous products may include CO₂ and CH₄ (Fu et al., 2010). These products might have resulted from carbon to carbon bond cracking reaction and gasification which are possible under the reaction conditions considered (Fu et al., 2010). Generally, the low molar yield of the target hydrocarbon product: heptadecane might be attributed to the formation of stearic acid, since platinum is very effective in hydrogenation reaction (Kitayama et al., 1997, Yoon et al., 1996), formation of side products from its cracking and catalyst deactivation. This low molar yield might also be due to formation of heavier molecules (C₄₀ – C₇₅) which are not easily detected by gas chromatography. This is possible since the presence of double bond in oleic acid has a tendency to support oligomerization reaction to heavier molecules (C₄₀ – C₇₅) (Alwan et al., 2015, Del Rio and Philp, 1992).

5.2.2.2.2 Effect of residence time on the products of hydrothermal decarboxylation of oleic acid using platinum on activated carbon (Pt/C) catalyst

Following the optimum temperature of 290 °C and the least molar yield of heptadecane of 10.74 % obtained at 330 °C after 4 h, from Section 5.2.2.2.1, we further explored the hydrothermal decarboxylation of oleic acid at the former temperature, in order to study the effect of residence time. The impact of varied residence time on yield and selectivity of the hydrocarbon products and the conversion of oleic acid were considered. The experiments were conducted as described in Section 2.7.4 using 100 mg of oleic acid, 20 mg of Pt/C catalyst, 15 mL of water and at varied residence times (0.5 and 1.0 h). The molar yield of the hydrocarbon products and the free fatty acids obtain are presented in Figure 5-17. The maximum values of

the molar yields of pentadecane and heptadecane were 4.6 % and 7.2 % obtained after 1 h and the minimum values were 4.0 % and 4.5 %, produced at 0.5 h. For palmitic acid, the maximum molar yield was 10.0 % and the minimum was 9.4 %, obtained at 1 h and 0.5 h, respectively.

The molar yield of stearic acid was 33.9 % after 0.5 h of the reaction and increased to 41.2 % at 1 h. The oleic acid decreased from 100 % before the reaction, to a mole percent of 33.9 % after 1 h, see Figure (5-17).

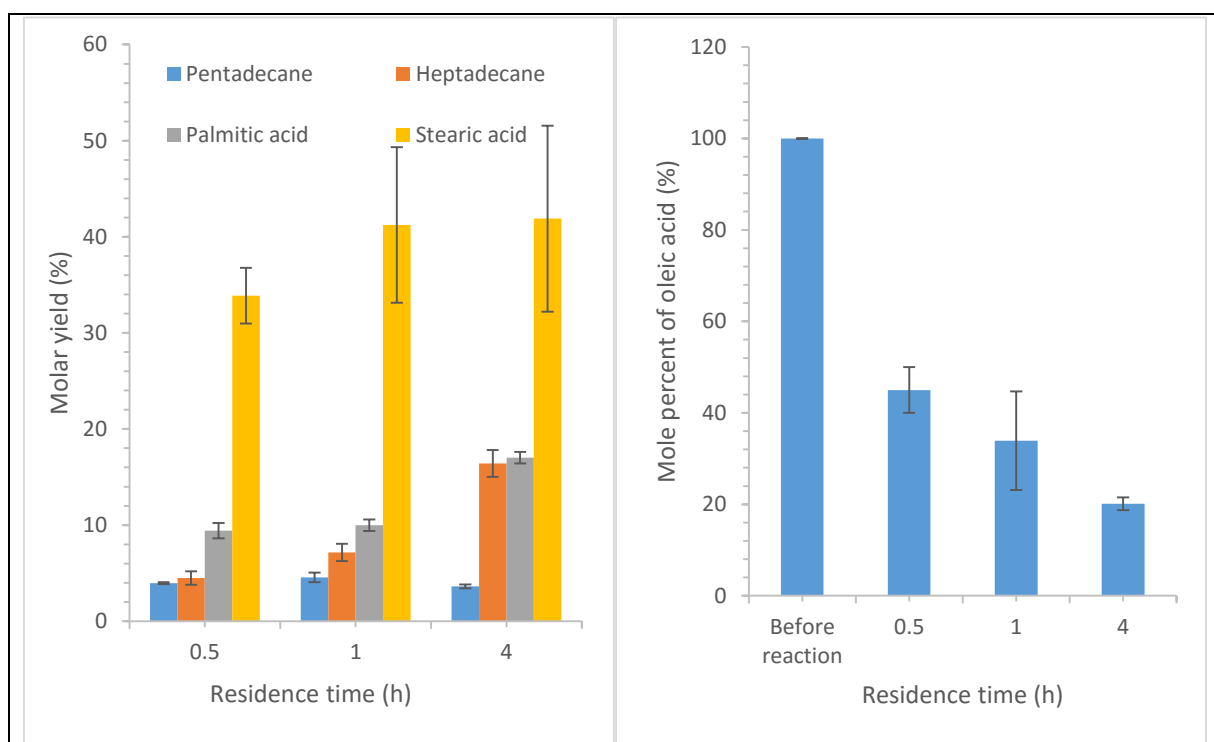


Figure 5-17: effect of residence time on molar yields of the hydrocarbon products from hydrothermal decarboxylation of oleic acid using platinum on activated carbon at a constant temperature of 290 °C

The results of the conversion of oleic acid and the selectivity of the hydrocarbons are presented in Figure 5-18. The lowest conversion was 53.5 % and the highest was 64.9 % after 0.5 h and 1 h, respectively. The maximum values of selectivity of pentadecane and heptadecane were 7.6 % and 11.0 %, obtained, after 0.5 h and 1 h, respectively. The minimum values were 7.0 % and 8.4 %, obtained after 1 h and 0.5 h, of reaction, respectively.

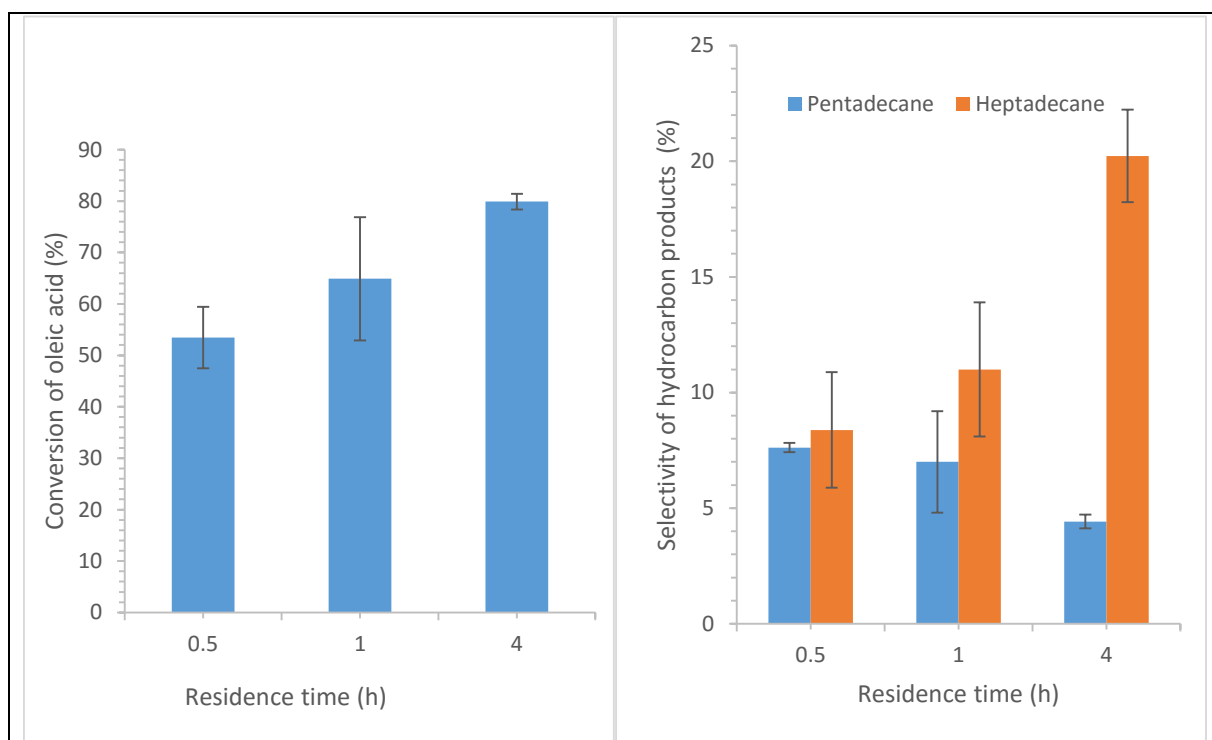


Figure 5-18: effect of residence time on conversion of oleic acid and selectivity of hydrocarbon products from hydrothermal decarboxylation at constant temperature of 290 °C using platinum on activated carbon catalyst. Where selectivity (%) = (number of moles of products recovered / (initial number of moles of reactant – number of moles of product recovered x 100)

The results have demonstrated that although, residence times (0.5 and 1 h) at constant temperature of 290 °C impacted the hydrocarbon products as was noticed in the increase in molar yields of pentadecane and heptadecane, the maximum values still remained those obtained at 290 °C after 4 h (Figures 5-17 and 5-18). As shown in Figures (5-17 and 5-18), the molar yields of heptadecane at 0.5 h and 1 h were 4.5 % and 7.2 %, respectively and their selectivities at these respective residence times were 8.4 % and 11.0 % at 290 °C. While after 4 h decarboxylation, as has also been presented in Section (5.2.2.2.1), the molar yield of heptadecane increased to 16.4% with a selectivity of 20.2 %. The low molar yields obtained at 0.5 h and 1 h relative to that from 4 h decarboxylation, could be attributed to the low conversion of oleic acid at these residence times to hydrocarbons. At these times: 0.5 h and 1 h, hydrogenation of oleic acid to stearic acid was more pronounced as seen by higher molar yields

of stearic acid, compared to decarboxylation which resulted to lower molar yields of hydrocarbons (pentadecane and heptadecane). While the higher molar yields of palmitic acid may have been the result of carbon to carbon cracking reaction (Fu et al., 2010). Generally, the reasons for low yield of hydrocarbons given in Section 5.2.2.2.1 may also be applied here.

In conclusion, the molar yield and selectivity of the main hydrocarbon product (heptadecane) of oleic acid decarboxylation increased with residence times (0.5, 1 and 4 h). The optimum residence time for the production of pentadecane was 1 h. The molar yields of palmitic and stearic acids, and conversion of oleic acid were found to increase with residence time.

5.2.2.2.3 *Effect of catalyst on yield and selectivity of hydrocarbons, and conversion of oleic acid using hydrothermal decarboxylation*

1. *Effect of catalyst and its type on yield and selectivity of hydrocarbons, and conversion of oleic acid using hydrothermal decarboxylation at constant temperature of 290 °C and residence time 4 h*

To assess the impact of catalyst on the performance of hydrothermal decarboxylation of oleic acid, experiments were conducted without (control) and with catalysts: Pt/C and Pd/C, at constant temperature of 290 °C for 4 h, as described in Section 2.7.4.1. The results of the molar yields and selectivities of hydrocarbons, and conversion of oleic acid are presented in Figure 5-19. As expected, the selectivity favoured the production of the primary hydrocarbon product: heptadecane. The molar yield of heptadecane increased from 1.9 % when the reaction was not catalysed to 16.4 % under the catalytic effect of Pt/C. The conversion of oleic acid when no catalyst was employed was 70.8 %, using Pd/C gave 73.3 % and with Pt/C resulted to 79.9 %. Thus, catalyst had an impact on the molar yield and selectivity of the hydrocarbon products and on the conversion of oleic acid, as observed by the molar yield of heptadecane which was about 5.63 – 8.78 times that obtained without catalyst. The effect of Pt/C on the reaction was greater than Pd/C, as illustrated in Figure 5-19.

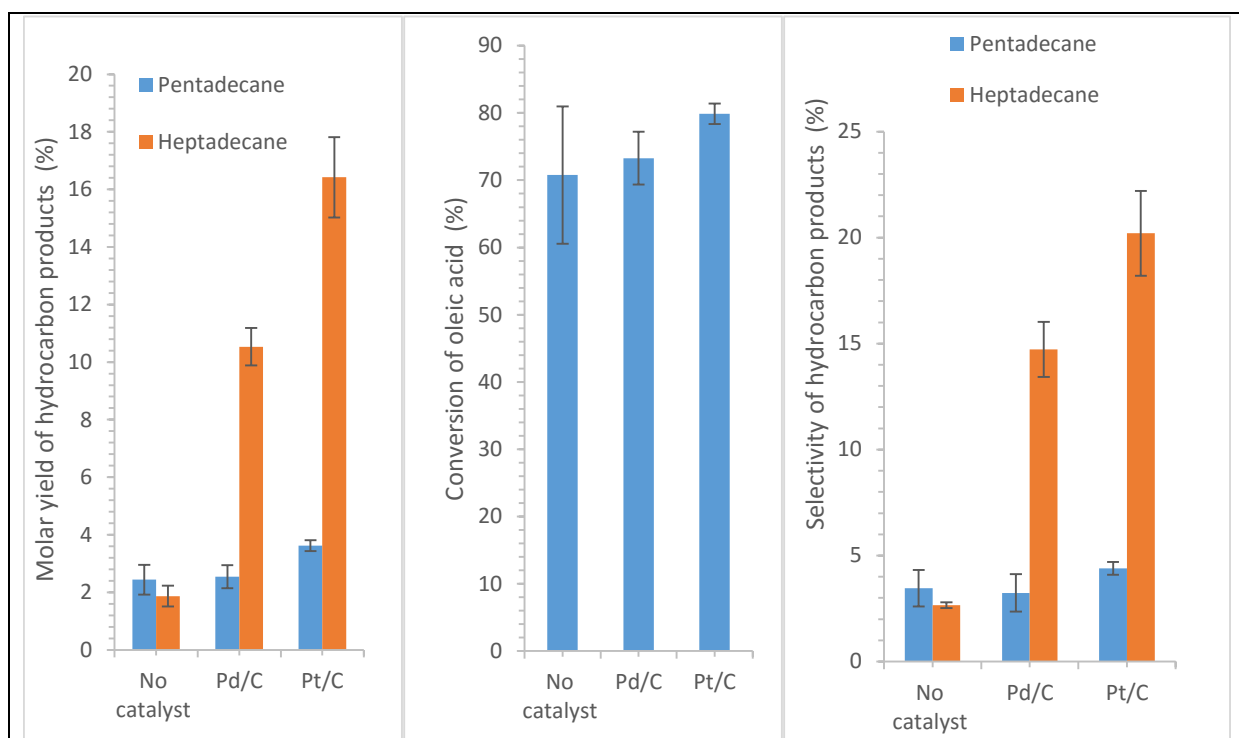


Figure 5-19: effect of catalyst on yield and selectivity of hydrocarbon products and conversion of oleic acid using hydrothermal decarboxylation at 290°C for 4h. Where selectivity (%) = (number of moles of products recovered / (initial number of moles of reactant – number of moles of product recovered x 100)

2. Effect of catalyst type on hydrocarbon yield from the hydrothermal decarboxylation of oleic acid at 290 °C and varied residence time

This was done to evaluate which catalyst (Pt/C and Pd/C) had greater impact on the molar yields of the hydrocarbon products (pentadecane and heptadecane) produced from the hydrothermal decarboxylation of oleic acid, under different residence times (0.5, 1 and 4 h), see Sections 5.2.2.1.2 and 5.2.2.2.2.

The highest molar yields of pentadecane: 4.4 % and 4.6 % were obtained after 1 h of decarboxylation, using Pd/C and Pt/C, respectively. Those of heptadecane were 10.5 % and 16.4 %, obtained using Pd/C and Pt/C, respectively at 4 h and constant temperature of 290 °C, see Section 5.2.2.2.3 (1) and Figure (5-20). Thus, catalyst influenced the hydrothermal

decarboxylation reaction of oleic acid to hydrocarbons (especially the main hydrocarbon product – heptadecane), with Pt/C showing a greater influence.

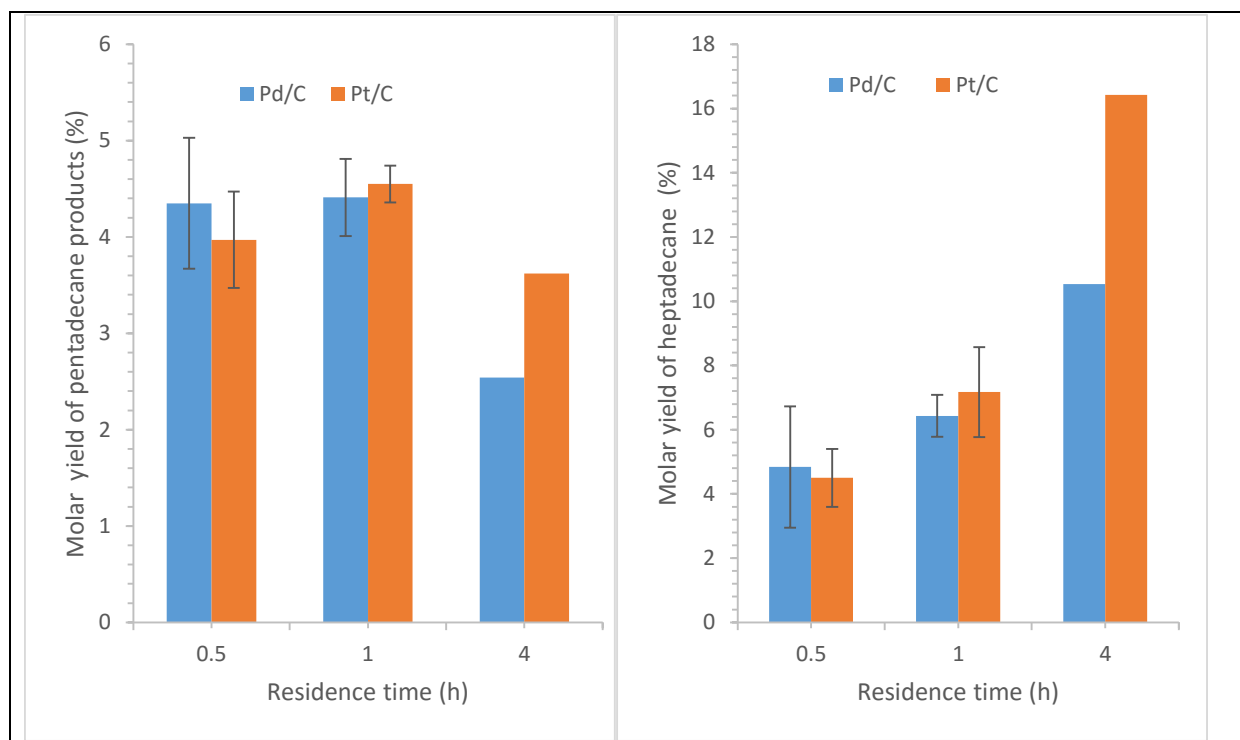


Figure 5-20: effect of catalyst type on yield of hydrocarbon products from the hydrothermal decarboxylation of oleic acid at 290°C and varied residence time

Generally, the results presented in Section 5.2.2.2.3 (1 and 2) show that catalysts: Pt/C and Pd/C influenced the performance of the hydrothermal decarboxylation of oleic acid. For instance, the molar yield of heptadecane was about 5.6 - 8.8 times that which was obtained without catalyst, at constant temperature of 290 °C and residence time of 4 h. Although, conversion of oleic acid upto 70.8% was achieved without catalyst, it appeared that due to higher molar yield of pentadecane (2.4%) than heptadecane (1.9 %) that most of the products produced were either converted to minor liquid products or gasified at the end of 4 h decarboxylation (Section 3.2.2.2.1). Using Pd/C catalyst increased the molar yields of the hydrocarbons: pentadecane (2.5 %) and heptadecane (10.5 %), and stearic acid (44.5 %), resulting from a higher conversion of oleic acid. Similarly, Pt/C increased the molar yields of

pentadecane (3.6 %), heptadecane (16.4%) and palmitic acid of 17.0 % due to increased conversion of oleic acid of 79.9 % with highest selectivity of 20.2 % favouring the production of the target hydrocarbon (heptadecane) (Figure 5-19). Comparatively, Pt/C was found to demonstrate higher catalytic activity than Pd/C. This is in agreement with the findings by Fu et al. (2010).

5.2.3 Composition of lipid extract from activated sludge

This was determined by gas chromatography without derivatization, according to the method described in Chapter 2.9.3, in order to know the type of hydrocarbons and the possible quality of the renewable diesel that will be produced from the hydrothermal decarboxylation reaction. The lipid composition obtained is presented in Figure 5-21, with the fatty acid profile showing a similarity with that shown in Chapter 2.2.3.3.2, but, different from those illustrated in Chapter 4.2.4.1.2 (1) in terms of the presence of other lipids: TAG, DAG, MAG, PC, PS, PI, palmitoleic acid, linoleic acid, behenic and decanedioc acid. These lipids appeared to be present in small quantities and thus, not visible under the scale used in plotting Figure 5-21. The difference in the time of sampling the activated sludge presumably contributed to the variation in the lipid composition. The results obtained indicate that the predominant fatty acids in the lipid extract were palmitic, oleic and stearic acids, and thus, pentadecane and heptadecane were expected to be in greater proportion compared to other potential hydrocarbons. The presence of high amounts of these fatty acids in the lipid extract, thus, informed the choice of palmitic acid and oleic acid as the model fatty acids. The results obtained are also discussed in this Section.

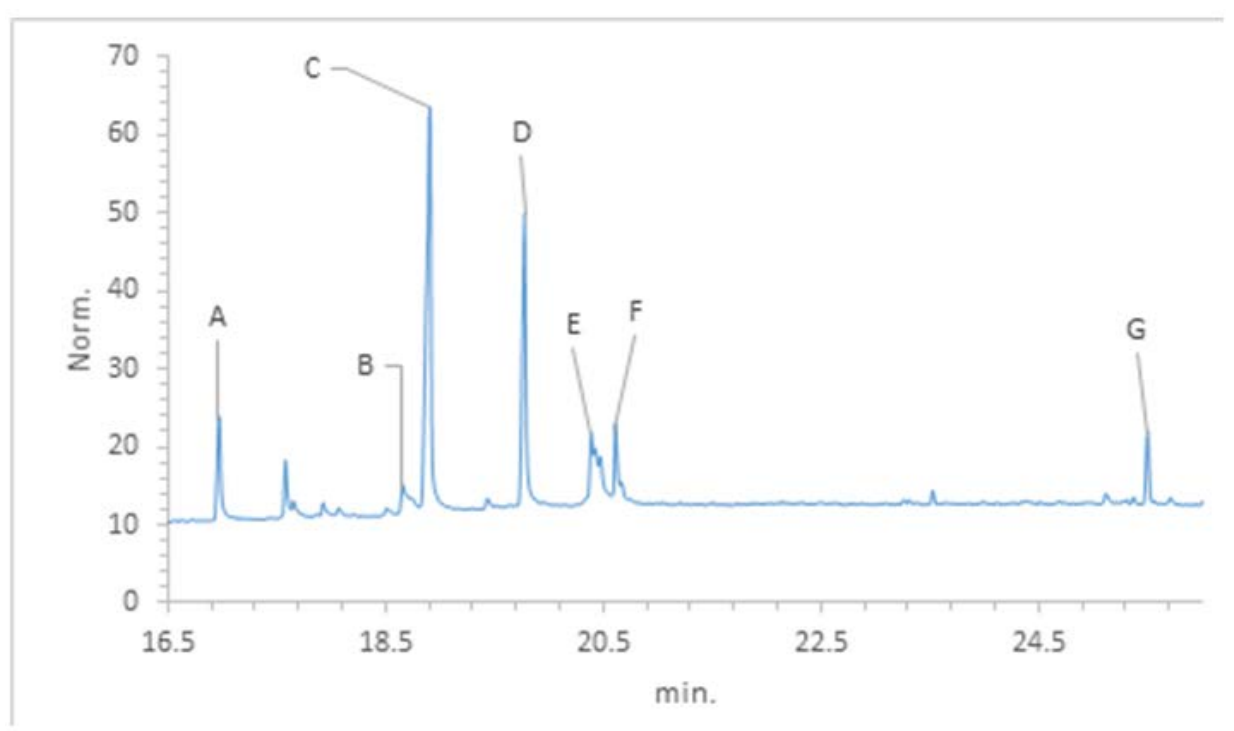
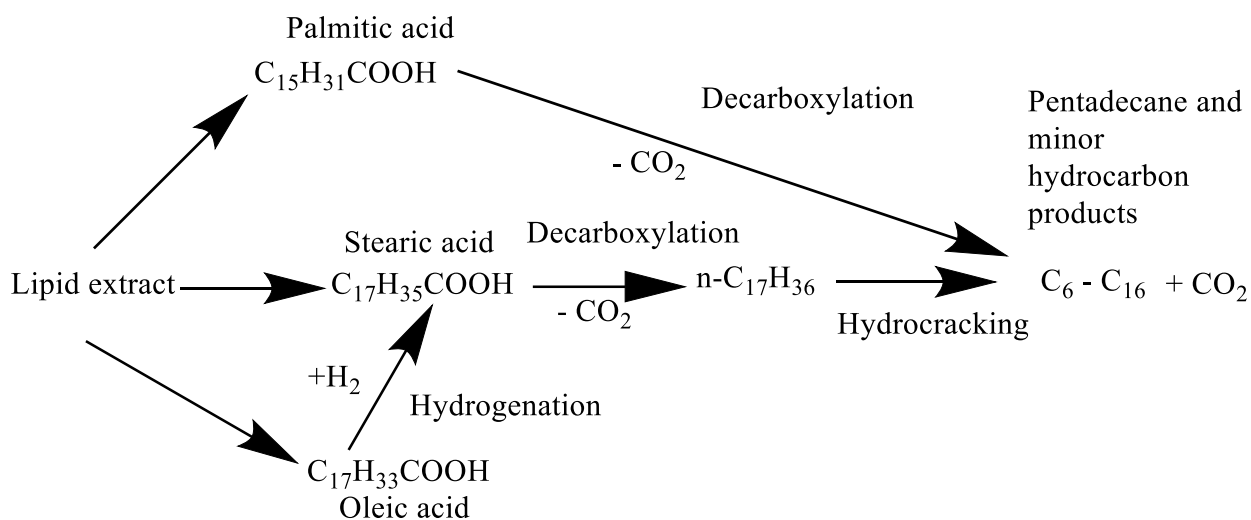


Figure 5-21: Gas chromatography analysis of lipid extract (A = unknown, B = cetyl palmitate, C = palmitic acid, D = heptadecanoic acid (internal standard), E = oleic acid, F = stearic acid and G = cholesterol). Lipid extract was obtained from Minworth STW, batch 2 activated sludge

5.2.4 Hydrothermal decarboxylation of lipid extract derived from activated sludge

This was carried out to assess the potential of catalytic hydrothermal decarboxylation of lipid derived from activated sludge in the production of renewable diesel or hydrocarbons. The catalysts used were palladium and platinum on activated carbons. The effect of these catalysts with respect to residence time are presented. Their efficacy was determined based on the yields of main hydrocarbons products: pentadecane and heptadecane produced. The suspected reaction mechanism is presented in Scheme 5-4. The reaction was carried out using three most prominent free fatty acids: palmitic, oleic and stearic acids identified from the activated sludge derived lipid (Figure 5-21). The reaction path for the conversion of palmitic and stearic acids to pentadecane and heptadecane, respectively, which are the main hydrocarbon products were by decarboxylation in the presence of Pd/C or Pt/C. That of oleic acid was by hydrogenation

leading to the production of stearic acid before decarboxylation to produce heptadecane, in the presence of Pd/C or Pt/C.



Scheme 5-4: scheme illustrating the suspected reactions involved in the hydrothermal decarboxylation of palmitic, oleic and stearic acids from activated sludge (Minworth STW, batch 2) derived lipid using Pd/C and Pt/C

5.2.4.1 Hydrothermal decarboxylation of lipid extract from activated sludge using palladium on activated carbon (Pd/C) catalyst

This was carried out to investigate the capacity of Pd/C catalyst in the hydrothermal decarboxylation of the lipid extract from activated sludge, leading to the production of renewable diesel/hydrocarbons. The effect of residence time was also investigated in order to obtain the optimum conditions which favour high yield of hydrocarbons (pentadecane and heptadecane). The experiments were conducted according to the procedures described in Section 2.7.4 using 100 mg of lipid extract, 20 mg of 5% Pd/C catalyst, 15 mL of water and residence times (0.5, 1, 3 and 4 h), at constant temperature of 290 °C. The plot of yields against the residence times are presented in Figure 5-22. Thus, Figure 5-22 shows that the maximum yields of pentadecane and heptadecane were 21.1 % and 11.9 %, obtained after 1 h of reaction. The minimum values were 9.8 % and 5.6 % obtained after 0.5 h, respectively. For the palmitic

acid (C16:0), the initial mole percent was 13.7 % before the decarboxylation reaction, this increased to a maximum of 25 %, after 1 h, before descending to 9.7 % at 4 h.

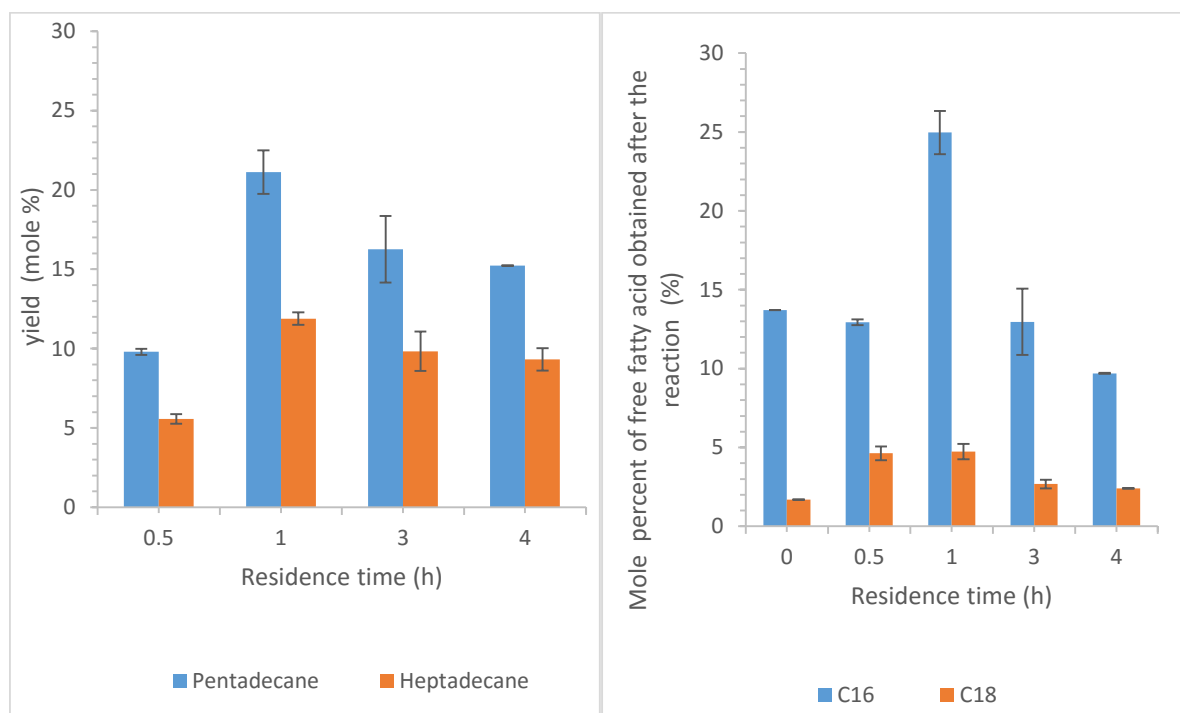


Figure 5-22: products of hydrothermal decarboxylation of lipid extract (from activated sludge obtained from Minworth STW, batch 2) using palladium on activated carbon catalyst at 290°C and varied residence time (C₁₆ and C₁₈ are free fatty, C₁₆ = palmitic acid, C₁₈ = oleic acid and stearic acid). The yields of pentadecane and heptadecane were determined based on the initial number of moles of C₁₆ and C₁₈ calculated from measured gas chromatography (GC) parameters.

Since the main hydrocarbon product of decarboxylation of stearic and oleic acids is heptadecane, we considered them as C₁₈ and used their initial number of moles in determining the yield of heptadecane produced from the reaction. The C₁₈ had a mole percent of 1.7 % before the start of the reaction, but, increased to a maximum value of 4.7 % at 1 h and then decreased to 2.4 at 4 h.

Considering the results presented above, catalytic hydrothermal decarboxylation of lipid derived from activated sludge is possible without the addition of hydrogen using palladium on activated carbon. The optimum conditions that produced a maximum yield of pentadecane

(21.1 %) and heptadecane (11.9 %) were temperature of 290 °C, residence time of 1 h, lipid extract of 100 mg, 20 mg of 5% Pd/C and 15 mL of water. Comparing these results with those obtained using the model fatty acids: palmitic and oleic acids, show that under the same operating conditions, higher molar yield of pentadecane (30.8 %) and lower molar yield of heptadecane (6.4 %) were produced, see Figures (5-3, 5-13 and 5-22). The higher molar yield of pentadecane produced using the model palmitic might be as a result of good contact with operating parameters, especially Pd/C catalyst and temperature, unlike the lipid extract containing other reacting species, which have a tendency of competing for these conditions. Similarly, the molar yield of the heptadecane was lower than that produced using the lipid extract, probably because of contributions from other reacting species in the lipid extract such as stearic acid, that have the tendency to produced the same primary product. It was observed that decarboxylating the lipid extract for long residence times of 3 and 4 h, decreased the palmitic acid and C₁₈, without a corresponding increase in the molar yields of pentadecane and heptadecane. As have explained previously, this might be due to catalyst deactivation, gasification and thermal cracking of more volatile substances (Section 5.2.1.2.3).

5.2.4.2 Hydrothermal decarboxylation of lipid extract using platinum on activated carbon (Pt/C) catalyst

This was conducted in order to investigate the potential of producing renewable diesel /hydrocarbons from lipid derived from activated sludge under a platinum on activated carbon catalysed hydrothermal decarboxylation. This was carried out at varied residence times (0.5, 1, 3, 4 h), 20 mg of 5% Pt/C, 100 mg of lipid extract and 15 mL of water, according to the procedures presented in Section 2.7.4. The effects of residence time on yield of hydrocarbon (pentadecane and heptadecane) are presented in Figure 5-23. The maximum yields of pentadecane and heptadecane were 23.2 % and 15.2 %, obtained after 1 h and the minimum values were 14.4 % and 6.4 % attained at 4 h, respectively. The mole percent of palmitic acid

increased from 13.7 % before the reaction to 19.0 %, before decreasing to 12.7 % at 4 h. That of C₁₈, increased from 1.7 % at time zero to 13.41%, at 0.5 h and then, descended to 3.0 %, after 3 h.

Following the results presented above, platinum on activated carbon efficiently catalysed the hydrothermal decarboxylation of lipid extract derived from activated sludge to hydrocarbons (pentadecane and heptadecane), with pentadecane as the most prominent. The optimum residence time was found to be 1 h, resulting to yield of pentadecane with 23.2 %. When the results were compared using those obtained from model palmitic acid and oleic acid under similar conditions, it was observed that the molar yields of pentadecane (31.8 %) was lower and heptadecane (7.2 %) was higher, see Figures (5-7 and 5-17). The reasons for these variations in molar yields might be the same with those given above (5.2.4.1).

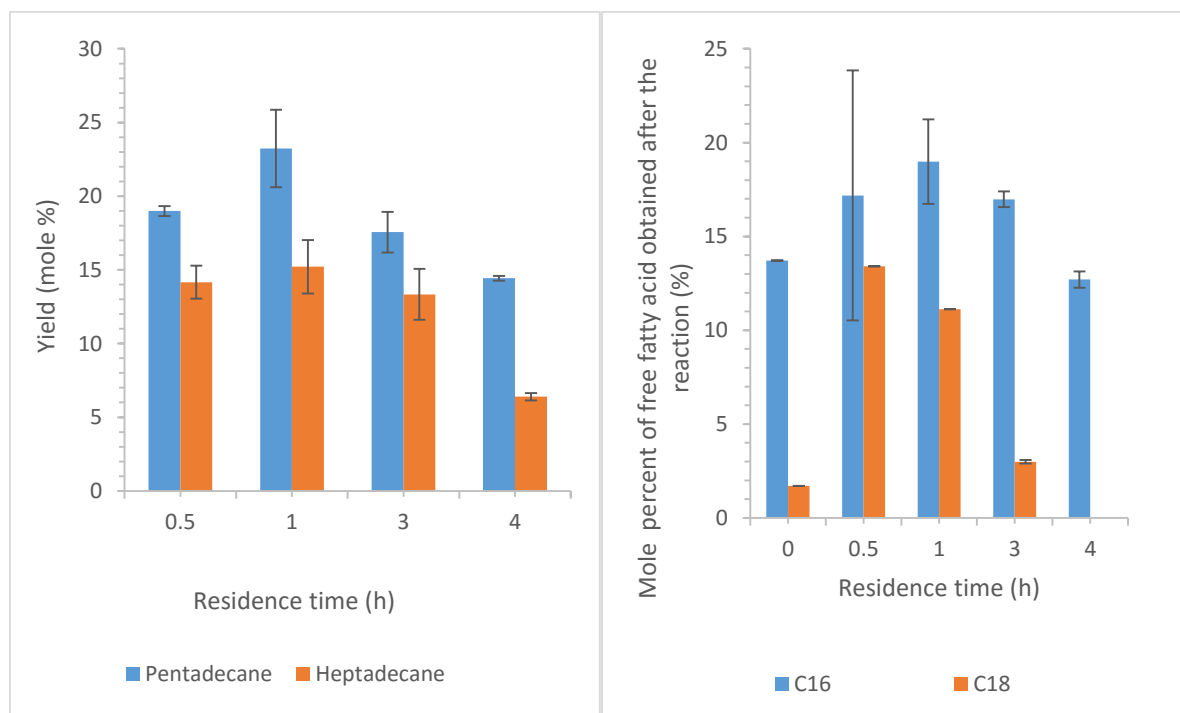


Figure 5-23: hydrocarbon products from the hydrothermal decarboxylation of lipid extract using platinum on activated carbon catalyst at 290 °C (C16 = palmitic acid, C18 = oleic acid and stearic acid). Note: at 4 h, there was no C₁₈ detected by the GC. This might probably be due to machine or experimental error. The lipid extract was obtained from activated sludge (Minworth STW, batch 2)

5.2.4.3 Effect of catalyst type on the yield of hydrocarbon product from the hydrothermal decarboxylation of lipid extracted from activated sludge

This was conducted to ascertain between the two catalysts: Pt/C and Pd/C which has a greater catalytic influence on the hydrothermal decarboxylation of lipid extracted from activated sludge, under different residence times (0.5, 1 and 3 h), at constant temperature of 290 °C. To achieve this, the yields of the hydrocarbons presented in Sections 5.2.3.2 and 5.2.3.3 were plotted with respect to residence time, as shown in Figure 5-24. At the optimum residence time of 1 h, the yields of pentadecane were 23.2 % and 21.1 %, obtained when the reaction was catalysed by Pt/C and Pd/C, respectively. Similarly, those of the heptadecane were 15.2 % and 11.9 %, obtained using Pt/C and Pd/C, respectively, as the catalysts, see Figure 5-24.

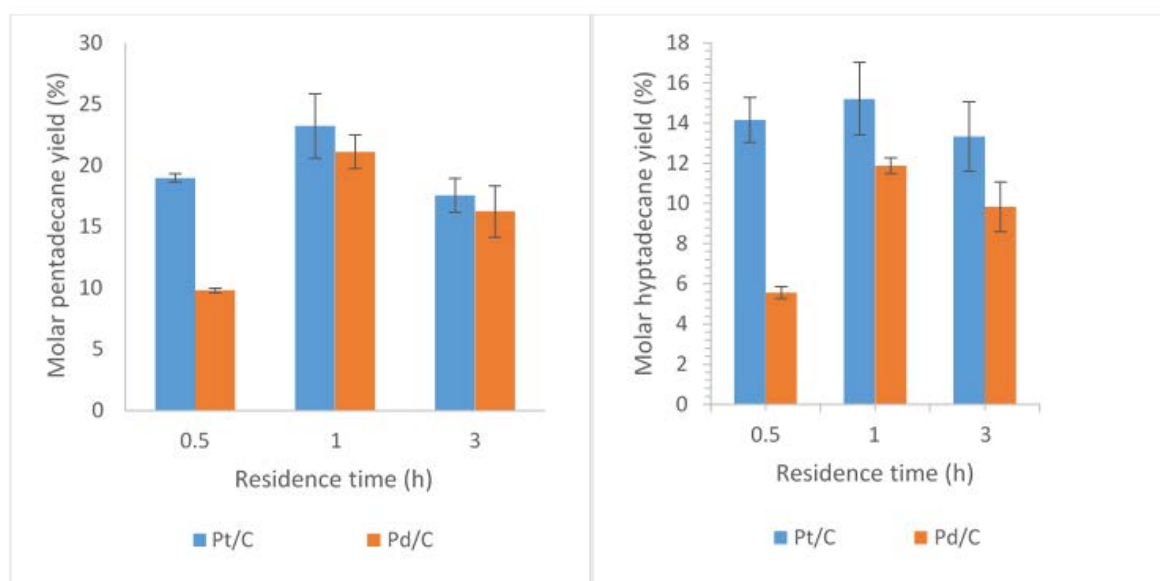


Figure 5-24: effect of catalyst type on the hydrocarbon yield of hydrothermal decarboxylation of lipid extract at 290°C (Pt/C = platinum on the activated carbon and Pd/C = palladium on activated carbon. Note: the molar yield was calculated based on the initial number of moles of the free fatty acids (C_{16} and C_{18}) obtained from the lipid derived from activated sludge (Minworth STW, batch 2).

Thus, based on the results presented above, Pt/C gave higher yields of hydrocarbons (pentadecane and heptadecane) compared to Pd/C, at the optimum residence time of 1 h,

constant temperature of 290 °C, 20 mg of 5% Pt/C, 100 mg of lipid extract and 15 mL of water. Hence, in order to maximum the yield of hydrocarbons/renewable diesel from activated sludge, platinum on activated carbon is recommended.

5.3 CONCLUSIONS

Hydrothermal decarboxylation of palmitic acid, oleic acid and lipid extract derived from activated sludge to produced hydrocarbons/renewable diesel, were feasible using 5% Pt/C and 5% Pd/C, under varied conditions of temperature and residence time. The primary hydrocarbon product of decarboxylation of palmitic acid and lipid extract was pentadecane and that of oleic acid was heptadecane. Platinum on activated carbon was found to show a greater catalytic effect than palladium on activated carbon. This was evidenced by the maximum molar yields of 55.3 % at 1.5 h and 23.3 % at 1 h, of pentadecane, produced from palmitic acid and lipid extract, and 16.4 % at 4 h and 15.2 % at 1 h, of heptadecane, obtained from oleic acid and lipid extract, respectively, at constant temperature of 290 °C, 20 mg of 5 % catalysts (Pt/C and Pd/C), 100 mg of fatty acids (palmitic acid, oleic acid and lipid extract) and 15 mL of water. The routes of the production of the hydrocarbons were decarboxylation (for pentadecane), and hydrogenation – decarboxylation (for heptadecane).

6 CHAPTER 6 CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

It has been illustrated that solid biomass content of the activated sludge slurry varied from one sewage works to another and depends on the season of collection. , as it is one of the factors which is usually considered when deciding on the feedstock for biodiesel production (Gui et al., 2008), except if the lipid yield is enhanced. For instance, the lipid yield obtained from the two sewage works was 2.8 – 7.4 (wt/wt) % using organic solvents. This was enhanced by the application of subcritical water to 18.2 ± 1.1 (wt/wt) % at 280 °C, using 5 % biomass loading and extraction time of 40 min. It further increased upon the use of optimization to 41.0 (wt/wt) under the operating conditions of 1 % biomass loading, temperature of 80 °C and extraction time of 20 min.

The conversion of the lipid extract obtained from the organic solvents to FAMES or biodiesel through methanolysis, using the operating conditions of residence time of 1 h, 3 mL of ~ 1.25 M HCl at 85 °C resulted to total yield of 13.9 (wt. %) or 0.4 (wt/wt, on dry activated sludge basis). This lipid yield (41.0 % wt/wt) corresponds to that which is obtained from rapeseed oil and greater than those from soybean oil (18 wt. %) and palm oil (36 wt. %), which are currently being used in the Europe, USA, and Malaysia, respectively, for biodiesel production (Korbitz, 1999, Singh and Gu, 2010, Yang et al., 2011). Hence, the lipid content in the activated sludge obtained in this work is presumably sufficient to support the industrial production of biodiesel.

The composition of the lipid extract from this activated sludge/solid biomass revealed the presence of lipid classes: sterol, wax ester, acylglycerol, free fatty acid and phospholipids. The

profile of the lipids remained unchanged upon the application of subcritical water treatment, but, the amounts were immensely influenced. The higher percentage of unsaturated fatty acid, indicated that lipid from activated sludge could be a good substrate for the production of biodiesel, as the tendency for formation of gel in the low temperature environment would be reduced. The dominant fatty acids were C₁₆ and C₁₈. The hydrothermal decarboxylation of these fatty acids at the optimum conditions of residence time of 1 h, catalyst loading of 20 mg and temperature of 290 °C gave maximum yields of the primary hydrocarbon products of 23.3 % (0.3 % of dry activated sludge) and 15.2 % (0.03 % of dry activated sludge) at 1 h of pentadecane and heptadecane, respectively, using Pt/C catalyst.

Generally, it has been demonstrated in this study that subcritical water treatment of activated sludge could enhance the lipid yield and hence, support the industrial production of bioenergy (biodiesel and renewable diesel) and oleochemicals, as solvent extraction alone does not give a full recovery. Comparatively, this current method of lipid enhancement, gave a higher yield with respect to those in the literature, obtained using sludge acidification, ultrasonic and supercritical carbon dioxide with or without additives (Dufreche et al., 2007, Olkiewicz et al., 2014)

6.2 RECOMMENDATIONS FOR FUTURE WORK

1. We have used hydrochloric acid to catalyse the methanolysis of lipid extract from activated sludge to FAMES. The problems associated with this method, otherwise known as chemical transesterification, include high energy demand, and uneasiness in recovery of catalyst and glycerol (Shimada et al., 2002, Tan et al., 2010). We recommend in order to reduce the effects of these problems that a future work on enzymatic methanolysis of activated sludge derived lipid be carried out using biocatalysts, such as, lipase. However, this process is also faced with challenges which include low conversion and rate of reaction, contamination of products and high cost

of lipase (Shimada et al., 2002, Yucel et al., 2012). Thus, comparatively, the cost of biodiesel production from sewage sludge by acidic catalysed in situ transesterification is less expensive than enzymatic methanolysis using lipase (Novozym ® 435) (Gerhard et al., 2015). This presumably is due to high cost of lipase and it can be overcome by using immobilised lipase which encourages the reusability of this enzyme and hence, enhancing the chances of industrial application (Yucel et al., 2012).

2. Sewage sludge according to Metcalf and Eddy (2003) contains some metals such as nickel and molybdenum. The commercial types of these catalysts have been used by Craig and Soveran (2001) to catalyse hydroprocessing of vegetable oil to hydrocarbons. For future work, we recommend that activated sludge should be investigated for in situ hydrothermal decarboxylation to hydrocarbons/renewable diesel, to evaluate the efficacy of these metals in catalysing the reaction.
3. The current work on hydrothermal decarboxylation and methanolysis of activated sludge derived lipid was carried out without using Design of Experiment for possible optimisation. We therefore, recommend that this be done, in order to achieve maximum yield of the hydrocarbons/renewable energy and FAMEs, respectively.

7 REFERENCES

- Abdeshahian, P., Dashti, M.G., Kalil, M.S., et al. (2010) Production of Biofuel using Biomass as a sustainable Biological Resource. **Biotechnology**, 9: (3): 274-282.
- Agarwal, A.K. (2007) Biofuels (alcohols and biodiesel) applications as fuels for internal combustion engines **Progress in Energy and Combustion Science**, 33: 233 - 271.
- Ahmadi, M., Macias, E.E., Jasinski, J.B., et al. (2014) Decarboxylation and further transformation of oleic acid over bifunctional, Pt/SAPO - 11 catalyst and Pt/chloride Al₂O₃ catalysts. **Journal of Molecular Catalysis A: Chemical**, 386: 14-19.
- Alwan, B.A., Salley, S.O. and Simon, N.K.Y. (2015) Biofuels production from hydrothermal decarboxylation of oleic acid and soybean oil over Ni-based transition metal carbides supported on Al-SBA-15. **Applied Catalysis A: General**, 498: 32-40.
- Anderson, M.J. and Whitcomb, P.J. (2005) **RSM Simplified: optimizing processes using response surface methods for design of experiments** United States of America Productivity Press, a division of Kraus Productivity Organization, Ltd
- Anderson, S. (ed.) (2004) **Soxtec: Its principles and applications**, Champaign: AOCS Press.
- AOAC (2005) Official Methods of Analysis of AOAC International **41.1.21 AOAC OFFICIAL METHOD 940.28 Fatty Acids (Free) in Crude and Refined Oils, First Action 1940, Final Action, AOAC International, USA**, 18th Edition.
- AOCS (1995) "Official and Tentative Methods of AOCS International ". 16 ed. Gaithersburg, USA, American Oil Chemist Society
- Arai, Y., Sako, T. and Takebayashi, Y. (2002) **Supercritical fluids: molecular interactions, physical properties and new applications**. London: Springer.

Arup, U., Ekman, S., Lindblom, L., et al. (1993) High Performance Thin Layer Chromatography (HPTLC), an Improved Technique for Screening Lichen Substances **Lichenologist** 25: (1): 61-71.

Atabani, A.E., Silitonga, A.S., Ong, H.C., et al. (2013) Non-edible Vegetable oils: A critical evaluation of oil extraction, fatty acid compositions, biodiesel production, characteristics, engine performance and emissions production. **Renewable and Sustainable Energy Reviews**, 18: 211-245.

Basu, P. (2010) **Biomass Gasification and Pyrolysis**. Elsevier Inc.

Basumatary, S. (2013) Non-Edible Oils of Assam as Potential Feedstocks for Biodiesel Production: A Review. **J. Chem. Bio. Phy. Sci.**, 3: (1): 551 - 558.

Becker, P., Koster, D., Popov, M.N., et al. (1999) The biodegradation of olive oil and the treatment of lipid-rich wool scouring wastewater under aerobic thermophilic conditions. **Water Res**, 33: 653-660.

Bermejo, M.D., Fdez-Polanco, F. and Cocero, M.J. (2006) Experimental study of the operational parameters of a transpiring wall reactor for supercritical water oxidation. **The Journal of Supercritical Fluids**, 39: 70-79.

Bezerra, M.A., Santelli, R.E., Oliveira, E.P., et al. (2008) Response surface methodology (RSM) as a tool for optimization in analytical chemistry **Talanta**, 76: 965-977.

Bien, J.B. (2007) "Sewage sludge. Theory and practice". Czestochowa, The University of Czestochowa.

Boda, L. Onyestyak, G., Solt, H., Lonyi, F., Valyon, J., Thernes, A. (2010) Catalytic hydroconversion of tricaprylin and caprylic acid as model reaction for biofuel production from triglycerides. **Appl Catal A**, 374:158 - 169.

- Boocock, D.G.B., Konar, S.K., Leung, A., et al. (1992) Fuels and chemicals from sewage sludge: 1. The solvent extraction and composition of a lipid from raw sewage sludge **Fuel**, 71: (11): 1283-1289.
- Borchardt, J.A., Cleland, J.K. and Redman, G.O. (1977) Viruses and Trace Contaminants in Waster and Wastewater. **Ann Arbor Science Publ., Ann Arbor.**
- Boukis, N., Galla, U., Diem, V., et al. (2004) Hydrogen production from biomass in supercritical water. **Chemical Engineering Transactions**, 4: 131.
- Brian, B.L. and Gardner, E.W. (1967) Preparation of Bacterial Fatty Acid Methyl Esters for Rapid Characterization by Gas-Liquid Chromatography **Applied Microbiology** 15: (6): 1499-1500.
- Bridgwater, A.V. (1999) Principles and practice of biomass fast pyrolysis processes for liquids. **J. Anal. Appl. Pyrolysis**, 51: 3-22.
- Bringe, N.A. (2005) "Soybean oil composition for biodiesel ". In Knothe, G.;Krahl, J. & Van Gerpen, J.H. (Eds.) **The Biodiesel Handbook** Champaign, IL, AOCS Press.
- Brondz, I. (2002) Development of fatty acid analysis by high performance liquid chromatography, gas chromatography and related techniques. **Anal. Chim. Acta**, 465: 1-37.
- Brunner, G. (2009) Near Critical and Supercritical water. Part 1. Hydrolytic and hydrothermal processes. **J of Supercritical Fluids**, 47: 373-381.
- Brunner, G., Misch, B., Firus, A., et al. (2001) "Cleaning of soil with supercritical water and supercritical carbon dioxide". In Stegmann, R.;Brunner, G.;Calmano, W. & Matz, G. (Eds.) **Treatment of Soil-Fundamentals, Analysis, Applications**. Berlin, Springer.
- Byers, J.A. (2003) Table values from phenomenex catalog. www.phenomenex.com, 9/14/2010 10:22 pm.
- CAMAG (2013) "Instrumental Thin-Layer Chromatography". Swiss, CAMAG.

- Carrapiso, A.I. and Garcia, C. (2000) Development in liquid analysis:some new extraction techniques and in situ transesterification. **Lipids**, 35: 1167-1177.
- Carvalho, G., Novais, J.M., Pinheiro, H.M., et al. (2004) Model development and application for surfactant biodegradation in an acclimatising activated sludge system. **Chemosphere**, 54: (10): 1495-1502.
- Casanova, I., Aggulo, L. and Aguado, A. (1997) Aggregate expansivity due to sulphide oxidation-II. **Physico-chem Syst Rate Model**, 26: (7): 1627-1632.
- Chan, K.W. and Ismail, M. (2009) Supercritical carbon dioxide fluid extraction of Hibiscus cannabinus L. seed oil: A potential solvent-free and high antioxidative edible oil. **Food Chemistry**, 114: 970 - 975.
- Chipasa, K.B. and Mdrzycka, K. (2008) Characterization of the fate of lipids in activated sludge. **Journal of Environmental Sciences**, 20: (5): 536-542.
- Christie, W.W. (1989) "The preparation of methyl esters and other derivatives". In Christie, W.W. (Ed.) **Gas Chromatography and Lipids**. 1st ed. Alloway, Scotland, Oily Press.
- Christie, W.W. (1993) "Preparation of Ester Derivatives of Fatty Acids for Chromatographic Analysis". In Christie, W.W. (Ed.) **Advances in Lipid Methodology** Two ed. Dundee, Oily Press
- Christie, W.W. (2003) **Lipid Analysis**. Bridge water, UK: Oily Press.
- Christie, W.W. (2011a) "Thin-Layer Chromatography of Lipids". In Harwood, J.I. & Weselake, R.J. (Eds.) **AOCS Lipid Library : Lipid Chemistry, Biology, Technology & Analysis** lipidlibrary.aocs.org, AOCS.
- Christie, W.W. (2011b) Waxes structure, composition, occurrence and analysis. **The lipid library**, April 28, 2011, <<http://lipidlibrary.aocs.org/lipids/waxes/index.htm>>.
- Clifford, T. (1999) **Fundamentals of supercritical fluids**. Oxford, UK: Oxford University Press.

Craig, W.K. and Soveran, D.W. (2001) "Production of hydrocarbons with a relatively high cetane rating". *In* U.S. (Ed.), 4, 992, 605.

Darnoko, D. (1999) **Continuous Production of Methyl Esters from Palm Oil and Recovery of Beta-Carotene by Membrane Technology**. Ph.D, University of Illinois.

David, F., Sandra, P. and Wylie, P. (2003) Improving the Analysis of Fatty Acid Methyl Esters Using Retention Time Locked Method and Retention Time Databases. **Agilent Technologie publication**, Application Note 5990-4822EN

(5988-5871 EN).

Davis, R.D. (1996) The impact of EU and UK environmental pressures on the future of sludge treatment and disposal. **Water Environ J**, 10: 65-69.

Dawidowicz, E.A. and Thompson, T.E. (1971) Artifacts produced by boron trifluoride methanolysis of a synthetic lecithin containing cyclopropane fatty acids (1-2-dihydrosterculoyl-3-sn-phosphatidycholine). **J. Lipid Res.**, 12: (5): 636-637.

Debruyne, J. and Hilborn, D. (2007) "Anaerobic Digestion Basics". **Factsheet**. Ontario, Ministry of Agriculture, Food and Rural Affairs.

Dejaegher, B. and Heyden, Y.V. (2011) Experimental designs and their recent advances in set-up, data interpretation, and analytical applications **Journal of Pharmaceutical and Biomedical Analysis** 56: 141-158.

Deka, D.C. and Basumatary, S. (2011) High quality biodiesel from yellow oleander (*Thevetia peruviana*) seed oil. **Biomass and Bioenergy**, 35: 1797 - 1803.

Del Rio, J.C. and Philp, R.P. (1992) Oligomerization of fatty acids as a possible source for high molecular weight hydrocarbons and sulphur-containing compounds in sediments. **Org. Geochem.**, 18: (6): 869 - 880.

Demirbas, A. (2006) Bio fuels sources, bio fuel policy, bio fuel economy and global bio fuel projections. **Energy Conversion and Management**.

- Demirbas, A. (2009) Biofuels: Securing the Planet's Future Energy Needs. **Springer-Verlag**.
- Do, P.T., Chiappero, M., Lobban, L.L., et al. (2009) Catalytic Deoxygenation of Methyl-Octanoate and Methyl-Stearate on Pt/Al₂O₃. **Catal Lett**, 130: 9-18.
- Dogru, M., Midilli, A. and Howarth, C.R. (2002) Gasification of sewage sludge using a throated downdraft gasifier and uncertainty analysis. **Fue Process Technol**, 75: 55-82.
- Dueholm, T.E., Andreasen, K.H. and Nielsen, P.H. (2001) Transformation of lipids in activated sludge. **Water Sci Technol**, 43: 165-172.
- Dufreche, S., Hernandez, R., French, T., et al. (2007) Extraction of Lipids from Municipal Wastewater Plant Microorganisms for Production of Biodiesel. **J Am Oil Chem Soc**, 84: 181 - 187.
- Dunford, N.T. and Temelli, F. (1997) Extraction conditions and moisture content of canola flakes as related to lipid composition of supercritical CO₂ extracts. **Journal of Food Science**, 62: 155 - 159.
- EC (2016) "Climate Action". **Paris Agreement**.
http://ec.europa.eu/clima/policies/international/negotiations/paris/index_en.htm, European Commission.
- Eisentraut, A. (2010) Sustainable production of second-generation biofuels: potential and perspectives in major economies and developing countries. **International Energy Agency**, 1-12.
- Fahy, E., Subramaniam, S., Brown, H.A., et al. (2005) A Comprehensive classification system for lipids. **Journal of Lipid Research**, 46: 839-861.
- FAO/WHO (1993) "Evaluation of certain food additives and contaminants". **Forty-first report of the joint FAO/WHO Committee on food additives**. Geneva, WHO.
- Fernando, B.D.C. (1994) **The use of steam treatment to upgrade lignocellulosic materials for animal feed**. Doctor of philosophy, Aberdeen.

- Ferraz, T.P.L., Fiuza, M.C., Santos, M.L.A.D., et al. (2004) Comparison of six methods for the extraction of lipids from serum in terms of effectiveness and protein preservation **J. Biochem. Biophys. Methods** 58: 187-193.
- Folch, J., Lees, M. and Stanley, G.H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. **Journal of Biological Chemistry**, 226: (1): 497-509.
- Fu, J., Lu, X. and Salvage, P. (2010) Catalytic hydrothermal deoxygenation of palmitic acid **Energy Environ. Sci.**, 2: 311-317.
- Fu, J., Lu, X. and Savage, P. (2011a) Hydrothermal Decarboxylation and Hydrogenation of Fatty Acids over Pt/C. **ChemSusChem**, 4: 481-486.
- Fu, J., Thompson, L.T., Lu, X., et al. (2011b) Activated Carbons for Hydrothermal Decarboxylation of Fatty Acids **ACS Catal.**, 1: 227-231.
- Furness, D.T. and Hoggett, L.A. (2000) Thermochemical Treatment of Sewage Sludge. **J. CIWEM**, 57-65.
- Fytili, D. and Zabaniotou, A. (2008) Utilization of sewage sludge in EU application of old and new methods-A review. **Renew.Sust.Energy.Rev.**, 12: 116-140.
- Galkin, A.A. and Lunin, V.V. (2005) Subcritical and supercritical water: a universal medium for chemical reactions. **Russ.Chem.Rev**, 74: 21-35.
- Garcia, A., Lucas, A.D., Rincon, J., et al. (1996) Supercritical carbon dioxide extraction of fatty and waxy material from rice bran. **Journal of American Oil Chemists Society**, 73: 1127 - 1131.
- Gerhard, N.S., Cea, M., Risco, V., et al. (2015) In situ biodiesel production from greasy sewage sludge using acid and enzymatic catalysts. **Bioresource Technology**, 179: 63 - 70.
- Giffin, K.M. and Wilson, W.H. (1996) Preparation and Analysis of FAMES by automated Esterification/Capillary GC. **Application N288-357**, Hewlett-Packard No. (23): 5965-1110E.

- Go, A., Liu, Y.T. and Ju, Y.H. (2014) Applicability of Subcritical Water Treatment on oil Seeds to Enhance Extractable Lipid. **Bioenerg. Res.**, 7: 711 - 719.
- Goh, C.S., Tan, K.T., Lee, K.T., et al. (2010) Bioethanol from lignocellulose: Status, perspectives and challenges in Malaysia. **Bioresour. Technol.**, 101: 4834-4841.
- Gomez, L.D., Clare, G.S. and MCQueen-Mason, J. (2008) Sustainable liquid biofuels from biomass: the writing's on the walls. **New Phytol**, 178: 473-485.
- Gui, M.M., Lee, K.T. and Bhatia, S. (2008) Feasibility of edible oil vs. non-edible oil vs. waste edible oil as biodiesel feedstock. **Energy**, 33: (11): 1646 - 1653.
- Gullison, R.E., Frumhoff, P.C., Canadell, J.G., et al. (2007) Tropical forests and climate policy. **Science**, 316: 985-986.
- Gunawan, F., Kurniawan, A., Gunawan, I., et al. (2014) Synthesis of biodiesel from vegetable oils wastewater sludge by in-situ subcritical methanol transesterification: Process evaluation and optimization. **Biomass and Bioenergy**, 69: 28 - 38.
- Gungoren, T., Saglam, M., Yuksel, M., et al. (2007) Near-Critical and Supercritical Fluid Extraction of Industrial Sewage Sludge. **Ind. Eng. Chem. Res.**, 6: 1051-1057.
- Han, D. (2012) **Concise Environmental Engineering**. PhD&Ventus Publishing Aps.
- Han, X., Cheng, L., Zhang, R., et al. (2009) Extraction of Safflower seed oil by supercritical CO₂. **Journal of Food Engineering**, 92: 370 - 376.
- Hanif, M., Atsuta, Y., Fujie, K., et al. (2010) Supercritical fluid extraction of microbial phospholipid fatty acids from activated sludge. **Journal of Chromatography A**, 1217: (43): 6704-6708.
- Hara, A. and Radin, N.S. (1978) Lipid Extraction of Tissues with a Low-Toxicity Solvent. **Analytical Biochemistry** 90: 420 - 426.
- Hass, M.J. and Foglia, T.A. (eds.) (2005) **Alternative feedstocks and technologies for biodiesel production**, Champaign: AOCS Press.

Henderson, R.J. and Tocher, D.R. (eds.) (1992) **Lipid Analysis: A Practical Approach** United States Oxford University Press.

Henze, M. (1992) Characterization of wastewater for modeling of activated sludge processes. **Water Sci Technol**, 25: 1-15.

Hibbert, D.B. (2012) Experimental design in chromatography: A tutorial review **Journal of Chromatography B**, 910: 2-13.

Higgins, A.J., Kaplovsky, A.J. and Hunder, J.V. (1982) Organic composition of aerobic, anaerobic, and compost-stabilized sludges. **Journal Water Pollution Control Federation**, 54: (5): 466-473.

Huynh, L., Do, Q., Kasim, N.S., et al. (2011) Isolation and analysis of wax esters from activated sludge. **Bioresource Technology**, 102: (20): 9518-9523.

Huynh, L., Kasim, N.S. and Ju, Y. (2010a) Extraction and analysis of neutral lipids from activated sludge with and without sub-critical water pre-treatment. **Bioresource Technology**, 101: (22): 8891-8896.

Huynh, L., Kasim, N.S. and Ju, Y.H. (2010b) Extraction and analysis of neutral lipids from activated sludge with and without sub-critical water pre-treatment. **Bioresource Technology**, 101: (22): 8891-8896.

Huynh, L., Nguyen, P.L.T., Ho, Q.P.H., et al. (2012) Catalyst-free fatty acid methyl ester production from wet activated sludge under subcritical water and methanol condition. **Bioresource Technology**, 123: 112-116.

Huynh, L.H., Kasim, N.S. and Ju, Y.H. (2010c) Extraction and analysis of neutral lipids from activated sludge with and without sub-critical water pre-treatment. **Bioresource Technology**, 101: (22): 8891-8896.

Hwu, C.S., Tseng, S.K., Yuan, C.Y., et al. (1998) Biosorption of long chain fatty acids in UASB treatment process. **Water Res**, 32: 1571-1579.

Ichihara, K. and Fukubayashi, Y. (2010) Preparation of fatty acid methyl esters for gas-liquid chromatography. **Journal of Lipid Research**, 51: 635-640

Ichihara, K., Waku, K., Yamaguchi, C., et al. (2002) A convenient method for determination of the C20-22 PUFA composition of glycerolipids in blood and breast milk. **Lipids**, 37: 523-526.

Ichihara, K., Yamaguchi, C., Nishijima, H., et al. (2003) Preparation of FAME from sterol esters. **J. Am. Oil. Chem. Soc.**, 80: 833-834.

Idesh, S., Kudo, S., Norinaga, K., et al. (2013) Catalytic Hydrothermal Reforming of Jatropha Oil in Subcritical Water for the Production of Green Fuels: Characteristics of Reactions over Pt and Ni Catalysts. **American Chemical Society** 27: 4796 - 4803

Inguanzo, M., Dominguez, A., Menendez, J.A., et al. (2002) On the Pyrolysis of sewage sludge: the influence of pyrolysis conditions on solid, liquid and gas fractions. **Journal of Analytical and Applied pyrolysis**, 63: 209-222.

Ishige, T., Tani, A., Sakai, Y., et al. (2003) Wax ester production by bacteria. **Curr. Opin. Microbiol**, 6: 244-442.

Issariyaku, T. and Dalai, A.K. (2014) Biodiesel from vegetable oils **Renewable and Sustainable Energy Reviews**, 31: 446-471.

Iverson, J., Lang, S.L.C. and Cooper, M.H. (2001) Comparison of the Bligh and Dyer and Folch methods for Total Lipid Determination in a Broad Range of Marine Tissue. **Paper no. L8731 in Lipids**, 36: 1283 - 1287.

Jaeger, M. and Mayer, M. (2000) The Noell conversion process-A gasification process for the pollutant-free disposal of sewage sludge and the recovery of energy and materials. **Water Sci Technol**, 41: (8): 37-44.

Jarde, E., Mansuy, L. and Faure, P. (2005) Organic markers in the lipidic fraction of sewage sludges. **Water Research**, 39: 1215-1232.

- Ju, H., Min, L. and Wenhui, G. (2000) Research Progress methods of lipids extraction from food. **He Bei Journal of Industrial Science & Technology**, 17: (1): 38-40.
- Kaiser, M.A. and Debbrecht, F.J. (eds.) (1977) **Qualitative and quantitative analysis by gas chromatography**, New York Wiley
- Kalnes, T., Marker, T. and Shonnard, D.R. (2007) Green diesel: A second generation biofuel. **International Journal of Chemical Reactor Engineering**, 5.
- Kalogo, Y. and Monteith, H. (2008) "State of Science Report: Energy and Resources Recovery from Sludge". Global Water Research Coalition
- Karayildirim, T., Yanik, J., Yuksel, M., et al. (2006) Characterisation of products from pyrolysis of waste sludge. **Fuel**, 85: 1498-1508.
- Kargbo, D.M. (2010) Biodiesel production from municipal sewage sludge. **Energy fuels review**, 24: 2791-2794.
- Karmakar, A., Karmakar, S. and Mukherjee, S. (2010) Properties of various Plants and animals feedstocks for biodiesel production, A review. **Bioresource Technology**, 101: 7201-7210.
- Katritzky, A.R. and Allin, S.M. (1996) Aquathermolysis: reactions of organic compounds with superheated water. **Accounts of Chemical Research**, 29: 399-406.
- Kent, J.A. (1992) **Riegel's Handbook of Industrial Chemistry**. Nine.New Tore: Van Nostrand Reinhold.
- Kharia, A.A. and Singhai, A.K. (2013) Screening of Most Effective Variables for Development of Gastroretentive Mucoadhesive Nanoparticles by Taguchi Design **ISRN Nanomaterials** 1-8.
- Khopkar, S.M. (2004) **Environmental Pollution Monitoring and Control**. New Delhi, New Age International

- Kitayama, Y., Muraoka, M., Takahashi, M., et al. (1997) Catalytic hydrogenation of linoleic acid over platinum-group metals supported on alumina. **American oil chemists society**, 74: (5): 525 - 529.
- Klopfenstein, W.E. (1971) On methylation of unsaturated acids using boron trihalide-methanol reagents **Journal of Lipid Research**, 12: 773 - 776.
- Knezevic, D., Van Swaaj, W.P.M. and Kersten, S.R.A. (2009) Hydrothermal conversion of biomass: I, Glucose Conversion in Hot Compressed Water **Ind Eng. Chem Res.**, 48: 4731-4743.
- Knothe, G. (2005) **What is biodiesel?** . 1st Champaign, Illinois: AOCS Press.
- Knothe, G. (2008) "Designer" Biodiesel: Optimizing Fatty Ester Composition to Improve Fuel Properties. **Energy & Fuels**, 22: 1358-1364.
- Knothe, G. (2010) Biodiesel and renewable diesel: A comparison. **Progress in Energy and Combustion Science**, 36: 364-373.
- Knothe, G., Krah, J. and Gerpen, J.V. (2005) "The biodiesel handbook". Champaign: AOCS Press.
- Kolattukudy, P.E. (1976) Introduction to Natural Waxes. **In: Kolattukudy, P. E.(Ed), Chemistry and Biochemistry of Natural Waxes**, 1-12.
- Korbitz, W. (1999) Biodiesel Production in Europe and North America: An Encouraging Prospect. **Renewable Energy**, 16: 1078 - 1083.
- Koritala, S. and Rohwedder, W.K. (1972) Formation of an artefact during methylation of conjugated fatty acids **Lipids**, 7: 274.
- Kritzer, P. (2004) Corrosion in high-temperature and supercritical water and aqueous solutions: a review. **J. Supercrit. Fluids**, 29: 1.

- Kronholm, J., Kalpala, J., Hartonen, K., et al. (2002) Pressurized hot water extraction coupled with supercritical water oxidation in remediation of sand and soil containing PAHs. **J. Supercrit. Fluids**, 23: 123-134.
- Kruse, A. (2008) Supercritical water gasification. **Biofuels, Bioproducts and Biorefining**, 2: 415-437.
- Kruse, A. (2009) Hydrothermal biomass gasification. **J. of Supercritical Fluids**, 47: 391-399.
- Kruse, A. and Dinjus, E. (2007) Hot compressed water as reaction medium and product: properties and synthesis reaction. **Journal of Supercritical Fluids**, 39: 362-380.
- Kruse, A. and Gawlik, A. (2003) Biomass Conversion in Water at 330 - 410 oC and 30 - 50 MPa. Identification of Key Compounds for Indicating Different Chemical Reaction Pathways **Ind. Eng. Chem. Res.**, 42: 267.
- Kulkarni, A.K., Daneshvarhosseini, S. and Yoshida, H. (2011) Effective recovery of pure aluminum from waste composite laminates by sub- and super-critical water. **The Journal of Supercritical Fluids**, 55: 992-997.
- Kulkarni, M.G., Gopinath, R., Meher, L.C., et al. (2006) Solid acid catalyzed biodiesel production by simultaneous esterification and transesterification **Green Chem**, 8: 1056-1062.
- Kumar, S. (2000) Technology options for municipal solid waste-to-energy project. **TERI Information Monitor on Environmental Science** 5, 1-11.
- Kuye, A.O. and Edeh, I. (2013) Production of bio oil from biomass using fast pyrolysis: A Critical Review. **JMR**.
- Lall, R.K., Proctor, A. and Jain, V.P. (2009) A Rapid, Micro FAME Preparation Method for Vegetable Oil Fatty Acid Analysis by Gas Chromatography. **J. Am. Oil. Chem. Soc.**, 86: 309-314.
- Lapuerta, M., Armas, O. and Garcia-Contreras, R. (2007) Stability of diesel-bioethanol blends for use in diesel engine. **Fuel**, 86: 1351 - 1357.

- Lebed, P.J., Potvin, S., Lariviere, D., et al. (2014) Optimization of solid phase extraction chromatography for the separation of Np from U and Pu using experimental design tools in complex matrices **Anal. Methods** 6: 139-146.
- Lee, S., Speight, J.G. and Loyalka, S.K. (2007) **Handbook of Alternative Fuel Technologies**. USA: CRC, Taylor and Francis Group.
- Lestari, S., Maki-Arvela, P., Simakova, I.L., et al. (2009) Catalytic deoxygenation of stearic acid and palmitic acid in semibatch mode. **Catal Lett**, 130: 48 - 51.
- Li, L., Coppola, E., Rine, J., et al. (2010) Catalytic Hydrothermal Conversion of Triglycerides to Non-esters Biofuels. **Energy Fuels**, 24: 1305-1315.
- Lili, L. and Shunsheng, C. (2008) Comparison on methods of extracting lipids from roes of *Carassius auratus*. **Journal Of Shanghai Fisheries University**, 17: (1): 38-40.
- Liu (1994a) Preparation of fatty acid methyl esters for gas-chromatographic analysis of lipids in biological materials. **J. Am. Oil. Chem. Soc.**, 71: 1179-1187.
- Liu, B. and Zhao, Z. (2007) Biodiesel production by direct methanolysis of oleaginous microbial biomass. **J Chem Technol Biotechnol**, 82: 775 - 780.
- Liu, K. (1994b) Preparation of fatty acid methyl esters for gas chromatographic analysis of lipids in biological materials. **J. Am. Oil. Chem. Soc.**, 71: (11): 1179-1187.
- Liu, Z., Mei, L., Wang, Q., et al. (2014) Optimization of subcritical fluid extraction of seed oil from *Nitraria tangutorum* using response surface methodology **LWT-Food Science and Technology** 56: 168-174.
- Luque de Castro, M.D. and Garcia-Ayuso, L.E. (1998) Soxhlet extraction of solid materials: an outdated technique with a promising innovative future. **Analytica Chimica ACTA**, 369: 1-10.
- Luthria, D.L. (2004) **Oil Extraction and Analysis: Critical issues and comparative studies**. AOCS: United States of America.

- Ma, F. and Hanna, M.A. (1999) Biodiesel production: a review. **Bioresource Technology**, 70: 1-15.
- Mahmood, T. and Elliott, A. (2006) A review of secondary sludge reduction technologies for the pulp and paper industry. **Water Research**, 40: (11): 2093-2112.
- Maki-Arvela, P., Rozmyslowicz, B., Lestari, S., et al. (2011) Catalytic Deoxygenation of Tall Oil Fatty Acid over Palladium Supported on Mesoporous Carbon. **Energy & Fuels**, 25: 2815 - 2825.
- Manara, P. and Zabaniotou, A. (2012) Towards sewage sludge based biofuels via thermochemical conversion-A review. **Renewable and Sustainable Energy Review**, 16: 2566-2582.
- Mangold, H.K. (ed.) (1969) **Thin Layer Chromatography** New York Springer
- Manirakiza, P., Covaci, A. and Schepens, P. (2001) Comparative Study on Total Lipid Determination using Soxhlet, Roese-Gottlieb, Bligh & Dyer, and Modified Bligh & Dyer Extraction Methods. **Journal of Food Composition and Analysis** 14: 93-100.
- Marchetti, J.M. and Errazu, A.F. (2008) Esterification of free fatty acids using sulfuric acid as catalyst in the presence of triglycerides. **Biomass and Bioenergy**, 32: (9): 892-895.
- Marrero, T.W., McAuley, B.P., Sutterlin, W.R., et al. (2004) Fate of heavy metals and radioactive metals in gasification of sewage sludge. **Waste Management**, 24: (193-8).
- Martinho, A., Matos, H.A., Gani, R., et al. (2008) Modelling and simulation of vegetable oil processes. **Food and Bioproducts Processing**, 86: 87 - 95.
- Massart, D.L., Vandeginste, B.G.M., Buydens, L.M.C., et al. (1997) **Handbook of Chemometrics and Qualimetrics: Part A** Amsterdam: Elsevier.
- Meher, L.C., Sagar, D.V. and Naik, S.N. (2006) Technical aspects of biodiesel production by transesterification: A review. **Renew. Sust. Energy Rev.**, 10: 248-268.

Meng, X., Yang, J., Xu, Z., et al. (2008) Biodiesel production from oleaginous microorganisms. **Renew Energy**, 34: 1-5.

Metcalf and Eddy (1991) **Wastewater engineering-treatment, disposal and reuse**. New York, USA: McGraw Hill.

Metcalf and Eddy (2003) "Wastewater Engineering, Treatment and Reuse". Fourth ed. New York, McGraw-Hill.

Mittal, A. (2011) Biological Wastewater Treatment. **Fulltide**, 31-44.

Mjos, S.A. and Waktola, H.D. (2015) Optimizing the relationship between chromatographic efficiency and retention times in temeparature-programmed gas chromatography **J. Sep. Sci.** , 0: 1-14.

Mondala, A., Liang, K., Toghiani, H., et al. (2009) Biodiesel production by in-situ transesterification of municipal primary and secondary sludges **Biores Tech** **100**, 1203-1210.

Monte, M.C., Fuente, E., Blanco, A., et al. (2008) Waste managmet from pulp and paper production in the European Union. **Waste Management doi:10.1016/j.wasman**, 02.002 (article in press).

Moore, A. (2008) Biofuels are dead Long live biofuels (?) B Part one. **New Biotechnol**, 25: 6-12.

Moquin, P.H.L. and Temelli, F. (2008) Kinetic modeling of hydrolysis of canola oil in supercritical media. **Fluids**, 45: 94-101.

Muhammad, H.A., Nabil, S.A., Alaadin, A.B., et al. (2002) Characterization, Utilization and Disposal of municipal sludge: The State of -the-art. **Arabian Journal for Science and Engineering**, 27: (1B): 3-27.

Myers, R.H., Montgomery, D.C. and Anderson, C.M. (1995) **Response Surface Methodology, Process and Product Optimization Using Designed Experiments**. New York Wiley

- Naik, S.N., Goud, V.V., Rout, P.K., et al. (2010) Production of first and second generation biofuels: A comprehensive review. **Renewable and Sustainable Energy Review**, 14: 578-597.
- Naude, Y., De Beer, W.H.J., Jooste1, S., et al. (1998) Comparison of supercritical fluid extraction and soxhlet extraction for the determination of DDT, DDD and DDE in sediment. **Water SA**, 24: 3.
- Nguyen, P.L.T., Go, A.W., Ayucitra, A., et al. (2013a) Production of biodiesel from activated sludge **Seminar Rekayasa Kimia Dan Proses**.
- Nguyen, P.L.T., Go, A.W. and Huynh, L.H. (2013b) A study on the mechanism of subcritical water treatment to maximize extractable cellular lipids **Biomass and Bioenergy**, 59: 532 - 539.
- Nigam, P.S. and Singh, A. (2011) Production of liquid biofuels from renewable resources. **Progress in Energy and Combustion Science**, 37: 52-68.
- Novak, J.T. and Kraus, D.L. (1973) Degradation of long chain fatty acids by activated sludge. **Water Res.**, 7: 843-851.
- OJL (1991) "Commission of European Communities, Council Directive 91/271/EEC of 21 May 1991 concerning Urban Wastewater Treatment". OJL 135, 30.5.1991, P. 40.
- Olkiewicz, M., Caporgno, M.P., Fortuny, A., et al. (2014a) Direct liquid-liquid extraction of lipid from municipal sewage sludge for biodiesel production **Fuel Processing Technology**, 128: 331-338.
- Olkiewicz, M., Fortuny, A., Stuber, F., et al. (2012) Evaluation of different sludges from WWTP as a potential source for biodiesel production. **Procedia Engineering s**, 42: 695-706.
- Olkiewicz, M., Fortuny, A., Stuber, F., et al. (2014b) Effects of pre-treatments on the lipid extraction and biodiesel production from municipal WWTP sludge. **Fuel**, 141: 250-257.

Ong, L.K., Effendi, C., Kurniawan, A., et al. (2013) Optimization of catalyst-free production of biodiesel from *Ceiba pentandra* (Kapok) oil with high free fatty acid contents. **Energy**, 57: 615 - 623.

Pagliero, C., Ochoa, N.A., Martino, P., et al. (2011) Separation of Sunflower Oil from Hexane by Use of Composite Polymeric Membranes. **J Am Oil Chem Soc**, 88: 1813 - 1819.

Passos, F., Garcia, J. and Ferrer, I. (2013) Impact of low temperature pretreatment on the anaerobic digestion of microalgal biomass. **Bioresource Technology**, 138: 79-86.

Pastore, C., Lopez, A., Lotito, V., et al. (2013) Biodiesel from dewatered wastewater sludge: A two-step process for a more advantageous production **Chemosphere**, 92: (667-673).

Patrick, H.R., Griffith, K., Liotta, C.L., et al. (2001) Near-critical water: a benign medium for catalytic reactions. **Industrial & Engineering Chemistry Research**, 40: 6063-6067.

Pauly, M. and Keegstra, K. (2008) Cell-wall carbohydrates and their modification as a resource for biofuels. **Plant J.**, 54: 559-568.

Pei, K.M. and Gaudy, A.F. (1971) Kinetic constants for aerobic growth of microbial populations selected with various single compounds and with municipal wastes as substrates. **Appl. Microbiol**, 21: 253-256.

Perry, R.H. and Green, D.W. (1999) **Perry's Chemical Engineers' Handbook**. 7.USA: McGraw-Hill.

Peterson, A.A., Vogel, F., Lachance, R.P., et al. (2008) Thermochemical biofuel production in hydrothermal media: a review of sub-and supercritical water technologies. **Energy & Environmental Science** 1: 32-65.

Pokoo-Aikins, G., Heath, A., Mentzer, R.A., et al. (2010) A multi-criteria approach to screening alternatives for converting sewage sludge to diesel. **Journal of Loss Prevention in the Process Industries**, 23: 412-420.

Quemeneur, M. and Marty, Y. (1994) Fatty acids and sterols in domestic wastewater. **Water Res**, 28: 1217-1226.

Quitain, A.T., Faisal, M., Kang, K., et al. (2002) Low-molecular-weight carboxylic acids produced from hydrothermal treatment of organic wastes. **J. Hazard. Mater**, 93: (2): 209-220.

Radmanesh, R., Courbariaux, Y., Chauki, Y., et al. (2006) A unified lumped approach in kinetics modelling of biomass pyrolysis. **Fuel**, 1211-1220.

Ragsdale and Associates (2012) **Water and Wastewater Technology Training** [online]. www.ragsdaleassociates.com Ragsdale and Associates [Accessed 19/11/12 2012]

Raunkjaer, K., Hvitved-Jacobsen, T. and Nielsen, P.H. (1994) Measurement of pools of protein, carbohydrate and lipid in domestic wastewater. **Water Sci Technol**, 41: 33-41.

Reddy, H.K., Muppaneni, T., Sun, Y., et al. (2014) Subcritical water extraction of lipids from wet algae for biodiesel production. **Fuel**, 133: 73-81.

Reichardt, C. (2003) **Solvents and Solvent Effects in Organic Chemistry**. Third. Wiley-VCH.

Reijnders, L. (2006) Conditions for the sustainability of biomass based fuel use. **Energy Policy**, 34: 863-876.

Revellame, E., Hernandez, R., French, W., et al. (2010) Biodiesel from activated sludge through in situ transesterification. **J. Chem. Technol. Biotechnol.**, 86: 61-68.

Revellame, E., Hernandez, R., French, W., et al. (2011) Production of biodiesel from wet activated sludge **J Chem Technol Biotechnol**, 86: 61-68.

Revellame, E.D., Hernandez, R., French, W., et al. (2012a) Lipid Storage compounds in raw activated sludge microorganisms for biofuels and oleochemicals production. **RSC Advances**, 2: 2015-2031.

Revellame, E.D., Holmes, W.E., Benson, T.J., et al. (2012b) Parameter Study on the Production of Renewable Fuels and Chemicals from Phospholipid-Containing Biomass **Top Catal**, 55: 185 - 195.

Robert, A.M. (1999) "Encyclopedia of Environmental Pollution and Cleanup

". **The Wiley Encyclopedia of Environmental Pollution and Clean Cleanup**. USA, John Wiley & Sons, Inc.

Rouser, G., Fleischer, S. and Yamamoto, A. (1970) Two Dimensional Thin Layer Chromatographic Separation of Polar Lipids and Determination of Phospholipids by Phosphorus Analysis of Spots. **Lipids**, 5: (5): 494-496.

Rouser, G., Kritchevsky, G., Yamamoto, A., et al. (1969) "Methods in Enzymology". In Lowenstein, J.M. (Ed.) New York Academic Press

Rozmyslowicz, B., Maki-Arvela, P., Lestari, S., et al. (2010) Catalytic Deoxygenation of Tall Oil Fatty Acids Over a Palladium-Mesoporous Carbon Catalyst: A New Source of Biofuels. **Top Catal**, 53: 1274 - 1277.

Rulkens, W. (2008) Sewage sludge as a Biomass Resources for the production of Energy: Overview and Assessment of the various options **American Chemical Society**, 22: 9 - 15.

Sahena, F., Zaidul, I.S.M., Jinap, S., et al. (2009) Application of Supercritical CO₂ in lipid extraction- A review. **Journal of Food Engineering**, 95: 240-253.

Şahinkaya, S. and Sevimli, M.F. (2013) Sono-thermal pre-treatment of waste activated sludge before anaerobic digestion. **Ultrasonics Sonochemistry**, 20: (1): 587-594.

Sambo, A.S. (2000) Strategic Developments in Renewable Energy in Nigeria. **International Association for Energy Economics**.

Savage, P.E. (1999) Organic Chemical Reactions in Supercritical Water. **Chem. Rev.**, 99: 603.

Schuchardt, U., Sercheli, R., Vargas, R. M. (1998) Transesterification of Vegetable Oils: a Review. **J.Braz. Chem. Soc**, 9(1): 199 - 210

Seltmann, G. and Holst, O. (2002) **The Bacteria Cell Wall**. Second ed. New York: Springer-Verlag.

Severn Trent Water (2015) Oral Communication

Sheppard, A.J. and Iverson, J.L. (1975) Esterification of fatty acids for gas-liquid chromatographic analysis. **J. Chromatogr. Sci.**, 13: 448-452.

Sherma, J. (2003) " Basic TLC Techniques, Materials, and Apparatus ". In Sherma, J. & Bernard, F. (Eds.) **Hand book of Thin-Layer Chromatography**. Revised and Expanded ed. New York Marcel Dekker, Inc. .

Shimada, Y., Watanabe, Y., Sugihara, A., et al. (2002) Enzymatic alcoholysis for biodiesel fuel production and application of the reaction to oil processing. **Journal of Molecular Catalysis B: Enzymatic**, 17: 133 - 142.

Siddiquee, M.N. and Rohani, S. (2011a) Experimental analysis of lipid extraction and biodiesel production from wastewater sludge. **Fuel Processing Technology** 92: 2241-2251.

Siddiquee, M.N. and Rohani, S. (2011b) Lipid extraction and biodiesel production from municipal sewage sludges: A review. **Renewable and Sustainable Energy Reviews**, 15: 1067-1072.

Sims, R., Taylor, M., Saddler, J., et al. (2008) From 1st - to - 2nd - generation biofuel Technologies: An overview of current industry and RD & D activities. **QECD/IEA**, 1-120.

Singh, B., Kumar, R. and Ahuja, N. (2005) Optimizing drug delivery systems using systematic "design of experiments". Part 1: fundamental aspects". **Critical Reviews in Therapeutic Drug Carrier Systems**, 22: (1): 27-105.

Singh, J. and Gu, S. (2010) Commercialization potential of microalgae for biofuels production. **Renew Sustain Energy Rev.**, 14: 2596 - 2610.

Siskin, M. and Katritzky, A.R. (2001) Reactivity of organic compound in superheated water: general background. **Chemical Reviews**, 101: 824-835.

Skartland, L.K., Mjos, S.A. and Grung, B. (2011) Experimental designs for modeling retention patterns and separation efficiency in analysis of fatty acid methyl esters by gas chromatography-mass spectrometry **Journal of Chromatography A**, 1218: 6823-6831.

Skipiski, U., Peterson, R.F., Sanders, J., et al. (1963) Thin-layer chromatography of phospholipids using silica gel without calcium sulfate binder. **J. Lipid Res.**, 4: 227-228.

Skipiski, V.P., Peterson, R.F. and Barclay, M. (1964) Quantitative analysis of phospholipids by thin layer chromatography **Biochem. J.**, 90: 374-378.

Smedes, F. and Askland, T.K. (1999) Revisiting the development of the Bligh and Dyer total lipid determination. **Mar. Poll. Bull.**, 38: 193-201.

Smith, B., Greenwell, H.C. and Whiting, A. (2009) Catalytic upgrading of tri-glycerides and fatty acids to transport biofuels **Energy Environ. Sci.**, 2: 262 - 271.

Snare, M., Kubickova, I., Maki-Arvela, P., et al. (2006) Heterogeneous Catalytic Deoxygenation of Stearic Acid for Production of Biodiesel. **Ind. Eng. Chem. Res.**, 45: 5708 - 5715.

Soriano, N.U., Migo, V.P. and Matsumura, M. (2006) Ozonized vegetable oil as pour point depressant for neat biodiesel **Fuel**, 85: 25-31.

Sovova, H. and Stateva, R.P. (2011) Supercritical fluid extraction from vegetable materials. **Rev Chem Eng**, 27: 79 - 156.

Spellman, F.R. (2009) **Hand of Water and Wastewater Treatment Plant Operations**. United States: Taylor & Francis Group, LLC.

Spuhler, D. (2010) **Sanitation System** [online]. URL Basel [Accessed 27.06.2014 2014]

Stolarek, P. and Ledakowicz, S. (2001) Thermal processing of sewage sludge by drying, pyrolysis, gasification and combustion. **Water Sci Technol**, 44: (10): 333-340.

Supelco (2014) Certificate of Analysis **Sigma-Aldrich Co. LLC**.

Tavakoli, O. and Yoshida, H. (2006) Conversion of scallop viscera wastes to valuable compounds using sub-critical water. **Green Chemistry**, 8: 100-106.

Telford, J.K. (2007) A Brief Intoduction to Design of Experiments **Johns Hopkins APL Technical Digest** 27: (3): 224-232.

Temelli, F. (2009) Perspectives on supercritical fluid processing of fats and oils **J. of Supercritical Fluids**, 47: 583 - 590.

Templeton, M.R. and Butler, D. (2011) "An Introduction to Wastewater Treatment". Ventus Publishing ApS.

Tixier, A.S.F., Elomri, A., Blanckaert, A., et al. (2011) Rapid and Green Analytical Method for the Determination of Quinoline Alkaloids from *Cinchona succirubra* Based on Microwave-Integrated Extraction and Leaching (MIEL) Prior to High Performance Liquid Chromatography **Int. J. Mol. Sci.**, 12: (11): 7846-7860.

Tsai, W.T., Lee, M.K. and Chang, Y.M. (2007) Fast pyrolysis of ricehusk: product yields and compositions. **Bioresour Technol**, 98: 22-28.

Tsigie, Y.A., Huynh, L., Ahmed, I.N., et al. (2012a) Maximizing biodiesel production from *Yarrowia lipolytica* Po 1g biomass using subcritical water pretreatment **Bioresource Technology**, 111: 201-207.

Tsigie, Y.A., Huynh, L.H., Ismadji, S., et al. (2012b) In situ biodiesel production from wet *Chlorella vulgaris* under subcritical condition **Chemical Engineering Journal**, 213: 104 - 108.

UNFCCC (2008) "Kyoto Protocol Reference Manual on Accounting of Emissions and Assigned Amount ". **United Nations Framework Convention on Climate Change**. Germany.

United-Nations (2003) "Waste-water Treatment Technologies: A General Review". New York, Economic and Social Commission for Western Asia.

Van Gerpen, J., Shanks, B., Prusko, R., et al. (2004) Biodiesel production technology. **NREL/SR-510-36244**.

Veeneman, R. (2011) Improving the Analysis of Fatty Acid Methyl Esters Using Automated Sample Preparation Techniques. **Agilent Technologies**, 1-8.

- Vinay, K.T., Sahoo, B.K., Anwar, A., et al. (2011) Fate of coliforms and pathogenic parasite in four full-scale sewage treatment systems in India. **Environ Monit Assess**, 181: 123-135.
- Vogel, A.I. (1956) **Practical Organic Chemistry** Third. Great Britain: Longmans.
- Vyas, A.P., Verma, J.L. and Subrahmanyam, V.N. (2010) A review on FAME production processes. **Fuel**, 89: 1-9.
- Wang, W.-C., Thapaliya, N., Campos, A., et al. (2012a) Hydrocarbon fuels from vegetable oils via hydrolysis and thermo-catalytic decarboxylation. **Fuel**, 95: 622-629.
- Wang, W., Thapaliya, N., Campos, A., et al. (2012b) Hydrocarbon fuels from vegetable oils via hydrolysis and thermo-catalytic decarboxylation. **Fuel**, 95: 622 - 629.
- Wang, Z. and Benning, C. (2011) Arabidopsis thaliana Polar Glycerolipid Profiling by Thin Layer Chromatography (TLC) Coupled with Gas-Liquid Chromatography (GLC). **Journal of Visualized Experiments JoVE**. 49: 1-6.
- Watanabe, M., Iida, T. and Inomata, H. (2006) Decomposition of a long chain saturated fatty acid with some additives in hot compressed water. **Energy Conversion and Management**, 47: 3344 - 3350.
- Watstech 2015 **Personal Communication**
- Weiner, F.W. and Matthew, R. (2003) "Environmental Engineering ". Edition ed. USA, Elsevier Science.
- Werle, S. (2015) Gasification of a Dried Sewage Sludge in a Laboratory Scale Fixed Bed Reactor. **Energies**, 8: 8562-8572.
- Werther, J. and Ogada, T. (1999) Sewage sludge combustion. **Progress Energy Combust Sci**, (25): 55-116.
- White, L.P. and Plaskett, L.G. (1981) **Biomass as fuel**. United States: Academic Press.
- Write, H.J., Segur, J.B., Clark, H.V., et al. (1944) A report on ester interchange **Oil and Soap** 21: 145-148.

- Wu, X., McLaren, J., Madl, R., et al. (2010) "Biofuels from Lignocellulosic Biomass". In Singh, O.V. & Harvey, S.P. (Eds.) **Sustainable Biotechnology**. Netherland, Elsevier.
- Xiao, L. (2010) **Evaluation of Extraction Methods for Recovery of Fatty Acids from Marine products**. Masters, University of Bergen.
- Xu, Z. and Godber, J.S. (2000) Comparison of supercritical fluid and solvent extraction methods in extracting Gammer - oryzanol from rice bran. **Journal of AOCS**, 77: 547 - 551.
- Yamaguchi, K., Murakami, M., Nakano, H., et al. (1986) Supercritical carbon dioxide extraction of oils from antarctic krill. **Journal of Agricultural and Food Chemistry**, 34: 904 - 907.
- Yaman, S. (2004) Pyrolysis of biomass to produce fuels and chemical feedstocks.
- Yan, J. and Lin, T. (2009) Biofuels in Asia. **Applied Energy**, 86: S1-S10.
- Yang, G., Zhang, G. and Wang, H. (2015) Current state of sludge production, management, treatment and disposal in China. **Water Research**, 78: 60-73.
- Yang, J., Xu, M., Zhang, X., et al. (2011) Lifecycle analysis on biodiesel from microalgae: water footprint and nutrient balance. **Renew Sustain Energy Rev.**, 102: 159-165.
- Yokoyama, S. (2008) "The Asian Biomass Handbook: A guide for Biomass production and utilization". In Ministry of Agriculture, F.a.F. (Ed.) Japan, Japan Institute of Energy.
- Yoon, C., Yang, M.X. and Somorjai, G.A. (1996) Hydrogenation of 1,3-butadiene on platinum surfaces of different structures. **Catal. Lett**, 46: 37-41.
- Yucel, S., Terzioglu, P. and Ozcimen, D. (2012) **Lipase Applications in Biodiesel Production**. Open Access: InTech.
- Yucheng, C. and Arkur, P. (2012) Sewage sludge-to-energy approaches based on anaerobic digestion and pyrolysis: Brief Overview and energy efficiency assessment. **Renewable and Sustainable Energy Review** 16: 1657-1665.

Zabaniotou, A., Loannidou, O. and Skoulou, V. (2008) Rapeseed residues utilization for energy and 2nd generation biofuels. **Fuel**, 87: 1492-1502.

Zhang, G., Hai, J., Ren, M., et al. (2013) Emission, mass balance, and distribution characteristics of PCDD/Fs and heavy metals during concombustion of sewage sludge and coal in power plants. **Environ. Sci. Technol**, 47: 2123-2130.

Zhu, F., Zhao, L., Jiang, H., et al. (2014) Comparison of the Lipid Content and Biodiesel Production from Municipal Sludge Using Three Extraction Methods **Energy & Fuels**, 28: 5277-5283.

(2012) **The 7th International Conference on Waste Management and Technology** Procedia Environmental Sciences 16

8 APPENDIX ANALYSIS OF LIPID EXTRACT FROM ACTIVATED SLUDGE USING THIN LAYER CHROMATOGRAPHY (TLC)

8.1 INTRODUCTION

Thin Layer Chromatography (TLC) is one of the oldest and most simple methods of analysing complex lipids, both qualitatively and quantitatively. It can also be used to monitor the process of a reaction and for the purification of a substance on the preparative scale. The features that made it possible for TLC to stand the test of time include easy applicability, high sensitivity, speed of separation, flexibility, versatility and cost effectiveness (Henderson and Tocher, 1992, Sherma, 2003).

The type of chromatography which is similar to TLC is the paper chromatography, although, differs in the nature of the stationary phase. TLC and paper chromatography comprise planar chromatography. Paper chromatography, although, can perform similar functions as the TLC, but the latter is faster, more effective, more appropriate for quantitative analysis and it also provides more opportunity to choose from a range of stationary and mobile phases (Sherma, 2003). Although, TLC and high-performance liquid chromatography (HPLC) are complementary, in separation and identification of substances due to the nature of their stationary phases, TLC is a manual technique while HPLC requires the use of pressure pumps and an on-line system (Sherma, 2003). TLC being a manual technique, with simple materials required, can easily being used for the qualitative analysis of complex lipids. The simple materials that are used in TLC include materials for sample application, development and detection.

TLC is quite versatile and renders itself to the analysis of different substances simultaneously, unlike HPLC and some other more sophisticated chromatographic techniques. Furthermore, the chromatograms produced from TLC can easily be visualized, stored for future references and further processed (Henderson and Tocher, 1992, Sherma, 2003).

Nevertheless, some of the drawbacks of TLC include poor reproducibility of the chromatograms, consumes a lot of time when large amount of the sample is to be applied on the plate, requires much volume of organic solvents with high risk to health and safety, and might give rise to less accuracy of quantitative results, since samples are spotted on the plate. However, all of these drawbacks of TLC can be tackled by the use of high performance thin layer chromatography (HPTLC) (Henderson and Tocher, 1992, Sherma, 2003). The high performance thin layer chromatography (HPTLC) is considered in Section 2.9.1

In the current investigation, TLC was employed as an analytical technique to carry out a qualitative analysis of the lipids extract from the activated sludge. The lipids were profiled into neutral and polar lipids (phospholipids).

8.2 MATERIALS AND METHODS

8.2.1 Qualitative analysis of lipids in the lipid extract from activated sludge using thin layer chromatography (TLC)

Sample, chemicals, materials and equipment/apparatuses

(i). Sample

This was the lipid extract obtained from activated sludge biomass (Finham STW, batch 2) using Folch method.

(ii). Chemicals

The organic solvents chloroform (reagent plus, 99.8), methanol (99 + %), hexane (99 pure, mixture of isomers), ethanol (analytical reagent grade), were purchased from Fisher Scientific

UK, respectively, petroleum ether (60 – 80 °C) from Fisons Scientific Equipment, acetone (purum, 99.0) and diethyl ether (for residual grade), from Sigma-Aldrich, respectively. Sulphuric acid (98%, analytical grade) was bought from BDH Limited Poole, England, acetic acid (purum) and ammonia solution (35%, analytical reagent grade), from Sigma-Aldrich. The neutral lipid standards: palmitic acid was bought from Cayman Chemical Company, oleic acid (99%), cholesterol (99 + %, GC), palmityl palmitate ($\geq 99\%$), cetyl palmitate from Sigma-Aldrich, respectively, mono-, di- and triglyceride mix from SUPELCO. The phospholipid standards: 1-monooleoyl – RAC – glycerol (C18:1, - CIS – 9), diolein (C18:1, (CIS) – 9) (dioleoylglycerol), glyceryl trioleate, L – α – phosphatidylcholine from egg yolk, type XV1-E ($\geq 99\%$, TLC, lyophilized powder), 1, 2 – diacyl-sn-glycero-3-phospho-L-serine ($\geq 97\%$ TLC), L- α -phosphatidylinositol from glycine max (soybean), phospholipid mixture (for HPLC from soybean) were purchased from Sigma-Aldrich, respectively. Primuline was bought from Sigma-Aldrich.

(iii). Materials

Hamilton syringe (170 2RN 25 μ L) was purchased from Sigma-Aldrich, pipette M1000 (fully adjustable, positive-displacement, 100 μ L to 1000 μ L), pipette M100 (fully adjustable, positive-displacement, 10 μ L to 100 μ L), pipette tip CP100 (X182 pipette tip CP100 racked sterile 100 μ L to 1000 μ L) and pipette tip CP100 (X192 pipette tip CP100 racked sterile 10 μ L to 100 μ L) were bought from Fisher Scientific UK, respectively. Thin layer chromatography plates (Partisil ®K6F, silica Gel 60 Å with fluorescent Indication, size (20 x 20 cm) was bought from Whatman International Ltd, England, layer thickness (250 μ m)), HPTLC silica gel 60 F₂₅₄S (50 glass plates, 20 x 10 cm, HX304850, Merck KGaA 64271 Darmstadt) was purchased from Sigma-Aldrich, screw top vial (4 mL, 15 x 45 mm, amber with white closed to cap, PTFE/Si Septa, 5183-4450 and 5183-4301), certified, pre-assembled screw top vial pack (2 mL, amber vials, PTFE/silicone/PTFE Septa, green caps, 5182-0556) were bought from Agilent Technologies LDA UK Limited, respectively and graduated conical tube amber and standard screw cap orange (2.0 mL tube: E1420-2337 Cap: E1480-0103) from Starlab (UK)

(iv). Equipment/apparatuses

Compact UV lamp (254nm uv/4 ~ watt, Science Company) was used to visualise the lipid spots for possible identification. TLC manual developing chamber (CAMAG) was used to provide

the enabling conditions for the separation of lipid components. Mettler-Toledo (AB204-S, Mettler-Toledo International Inc.) was used to measure the weight of lipids. Memmert Oven (AtmosSAFE) was used for providing the required temperature for charring the derivatized TLC plate. Re-circulating Filtration Fume Cupboard (Astecair 5000E, SN: 0322 Carbon filter type AC/AMN BLEND, Willaire Scientific Company) was used to ensure health and safety.

8.2.1.1 *Experimental procedures*

The TLC plate was dimensioned horizontally (1.5 cm and 1 cm from bottom and top, respectively) and vertically (interval of 2 cm) to ensure that lipids do not dissolve in the solvent system prior to elution and that they were well-spaced from each other. After which, it was prewashed in the TLC plate Pre-wash Chamber (lined with four adsorbent papers) which was earlier saturated with chloroform/methanol (50/50, v/v) to remove any impurities which could interfere with visualization process (Henderson and Tocher, 1992). At the completion of this, the TLC plate was allowed to dry in the TLC Plate Storage Chamber inside the fume cupboard and further activated in the oven at 120 °C for 2.5h before allowing it to cool down for 10 min. This was followed by lipid loading which was applied using Hamilton syringe at least in triplicate at the middle of the vertical lines on the baseline of the plate, after which they were eluted, then detected.

8.2.1.1.1 *Optimising the operating parameter for TLC analysis of lipid*

Factors such as the carrier solvent for lipids, solvent system, charring temperature, charring time, derivatization reagents, activation of the TLC plate and detection methods tend to affect the quality of the results produced from TLC. Based on this, we investigated the optimum conditions which could enhance the production of good quality chromatograms, using TLC.

(1). Carrier solvent for the dissolution of lipids

A suitable solvent system was identified in order to ensure that the lipid was well dissolved before loading it on the TLC plate. This was done by preparing 40 mg/mL of lipid extract in 1 mL chloroform/methanol (2/1, v/v) and 30 mg/mL of each lipid standard (palmitic acid and cholesterol) in 1 mL hexane, diethyl ether, methanol or chloroform/methanol (2/1, v/v). Thereafter, 0.15 mg of each lipid was applied on the TLC plate and analysed, according to the method described in Section 8.2.1.1.

(2). Determining the effect of plate activation on the quality of the TLC result

This was done using two TLC plates, marked A and B, respectively. Plate A was activated in the Memmert oven at 120°C for 2h while plate B was not activated. The procedure described in Section 8.2.1.1 was applied while loading 0.4 mg of lipid extract, lipid standards: cholesterol 0.15 mg and triacylglycerol 0.15mg on the plate and eluting using hexane/diethyl ether/glacial acetic acid (70/30/3, v/v/v). The derivatizing reagent was 5% sulphuric acid in ethanol, charring temperature was 140°C and that of time was 40 min.

(3). Determining a suitable detecting method for lipid identification

This involved determining whether non-destructive or destructive method of lipid detection was suitable for the TLC analysis. In using a destructive method, suitable charring conditions (temperature and time) and derivatizing reagent were determined while primuline was used as a visualising reagent under a UV lamp for the non-destructive method.

(3a). Destructive method of lipid detection

(i). Determining optimum charring conditions

The procedure described in Section 8.2.1.1 was applied using four TLC plates. Plate 1 was loaded with 0.15 mg of palmitic acid, cholesterol 0.15 mg, glyceryl trioleate 0.05 mg and lipid

extract 0.4 mg. Plate 2 was loaded with palmitic acid 0.15 mg and lipid extract 0.4 mg. While plates 3 and 4 were loaded with mono-, di- and triacylglycerol mix 0.2 mg and palmityl palmitate 0.24 mg, cholesterol 0.15mg and lipid extract 0.4 mg. Plates 1 and 2 were developed using hexane/diethyl ether/acetic acid (70/30/1, v/v/v) and 3 and 4 were eluted using petroleum ether/diethyl ether/acetic acid (80/20/1, v/v/v). The lipids on the plates were derivatized by spraying them with 5% concentrated sulphuric acid in ethanol. After which, they were charred at varied temperatures 140 - 180°C and times 5 – 120 min for detection.

(ii). Identifying a suitable derivatizing reagent

The procedure presented in Section 8.2.1.1 was used while applying 28.72 µg of phospholipid standard mix and 240 µg of lipid extract on the two TLC plates A and B. The lipid application was done in triplicate per plate and each plate was developed using chloroform/methanol/28% ammonia (65/25/5, v/v/v). The lipid on plate A was derivatized by spraying it with 5% sulphuric acid (98%) in ethanol and that of plate B with 5% sulphuric acid (98%) in methanol. The plates were charred in the oven at 180°C for 10 min for the detection of the lipid.

(3b). Non-destructive method of lipid detection

The non-destructive method was done by spraying the developed TLC plate with a visualizing reagent 5 mg of primuline in 100 mL acetone/water (80/20, v/v) and then viewing the TLC plate under a UV lamp with a wavelength of 254 nm.

(4). Choice of the eluent solvent/solvent system

This was made by evaluating the capacity of the eluent solvent or solvent system in resolving the lipid compounds, using the method described in Sections 8.2.1.1.2 and 8.2.1.1.3 for both neutral and phospholipids, respectively.

8.2.1.1.2 Identifying the neutral lipid in the activated sludge

The neutral lipids in the activated sludge were identified according the procedure described in Section 8.2.1.1. The lipid extract and standards were dissolved in chloroform/methanol (2/1, v/v), spotted on the baseline of the four TLC plates, at the points marked A, B, C, D, E, F, G and H and eluted using solvent systems, as presented in Table 8-1. The lipids were detected using 5% sulphuric acid (98%) in ethanol and charred at 180°C for 10min except palmitic acid that was baked at 180°C for 60 min. Each lipid was eluted at least eight times and the best plates selected and combined together for presentation.

Table 8-1: operating conditions for the TLC analysis of lipids extract from the activated sludge (Finham STW, batch 2)

Plates	Letters on the baseline	Lipid standards/ lipid extract	Solvent system	Amount loaded on the plate (µg)
1	A	Lipid extract	Petroleum ether	400
	B	Mono acylglycerols, Mono-, di- (1, 2-diacylglycerol and 1, 3-diacylglycerol) and triacylglycerol mix	/diethyl ether/ acetic acid (80/20/1, v/v/v)	200
	C	Cholesterol		152
	D	Palmityl palmitate		238
	E	Lipid extract		400
2	F	Mono-, di- (1, 2-diacylglycerol and 1,3-diacylglycerol) and triacylglycerol mix	Hexane/diethyl ether/ acetic acid (70/30/1, v/v/v)	200
	G	Lipid extract		400
3	H	Palmitic acid	Hexane/diethyl ether/ acetic acid (70/30/3, v/v/v)	150

8.2.1.1.3 Identifying the phospholipids in activated sludge

This was done with TLC according to the procedure presented in Section 8.2.1.1. The amount of lipid extract applied on the plate was 240 µg and that of phospholipid standards were 0.44µg

L- α – phosphatidylethanolamine (PE), 0.69 μg L- α – phosphatidylcholine (PC), 24.84 μg L- α – phosphatidylinositol (PI) and 2.75 μg L- α lysophosphatidylcholine (LPC). The solvent systems used for the lipid separation were chloroform/methanol/acetic acid/water (25/15/4/2, v/v/v/v) (V. P. Skipski et al., 1964), chloroform/methanol/acetic acid/water (50/25/7/3, v/v/v/v) (U. Skipski et al., 1963), chloroform/acetone/methanol/acetic acid/water (6/8/2/2/1, v/v/v/v) (Rouser et al., 1970), chloroform/methanol/28% ammonia (65/25/5, v/v/v) (Rouser et al., 1970). The lipids were detected using the destructive method by spraying with 5% sulphuric acid (98%) in ethanol and charring at 180°C for 10 min.

8.3 RESULTS AND DISCUSSIONS

8.3.1 *Optimising the operating parameters for TLC analysis of lipid extract from activated sludge*

(i). Carrier solvent for dissolution of lipids

The choice of carrier solvent for dissolving lipid prior to applying it on the TLC plate was important to ensure that accurate loading was achieved. This was determined following an analysis carried out using TLC, according to the method described in Sections 8.2.1.1.1 (1). The results show that although, all the solvents (hexane, diethyl ether, methanol and chloroform/methanol (2/1, v/v) dissolved both lipid extract and standards, chloroform/methanol (2/1, v/v) gave a higher resolution of lipid spots after separation. This is probably, because, that mixture is known to dissolve both neutral and polar lipids. This solvent mixture was also used to carry out the solvent extraction of lipids. Hence it was preferred to other organic solvents considered and used in the TLC and high performance thin layer chromatography (HPTLC), see Figure 8-1, Sections 2.9.1 and 3.2.3.1.

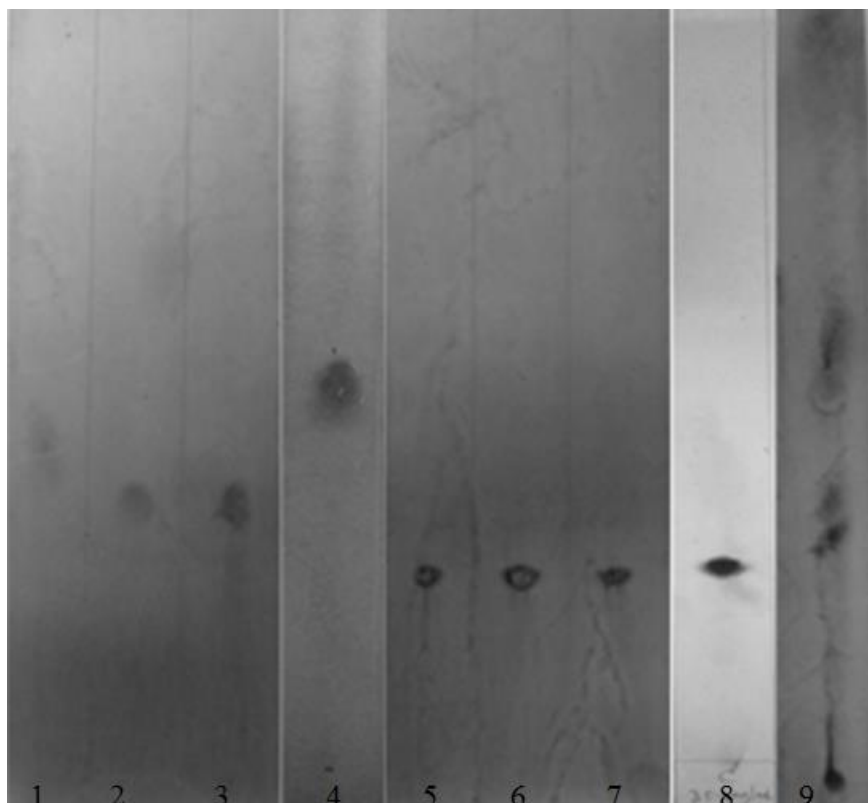


Figure 8-1: an illustration of a TLC chromatograms of lipid extract and standards eluted using hexane/diethyl ether/glacial acetic acid (70/30/3, v/v/v) (lanes 1, 2, 3, 4 = palmitic acid dissolved in hexane, diethyl ether, methanol and chloroform/methanol (2/1, v/v), lanes 5, 6, 7 and 8 = cholesterol dissolved in hexane, diethyl ether, methanol and chloroform/methanol (2/1, v/v) and lane 9 = lipid extract dissolved in chloroform/methanol (2/1, v/v). The lipid extract was obtained from Finham STW, batch 2.

(ii) Determining the effect of plate activation on the quality of the TLC result

An investigation was made to determine if there was any impact of activating the TLC plate on the lipid separation and identification. This was done according to the method described in Section 8.2.1.1.1 (2)). The results show that lipid extract was not well separated when non-activated plate (plate A) was used, but, more distinct separation of lipid spots were observed with the activated plate (plate B) (Figure 8-2). This might be due to the presence of adsorbed moisture on the non-activated plate which tends to pose a resistance to the capillary movement of lipids in the solvent system during chromatographic process (Wang and Benning, 2011). Therefore, there was need to activate the TLC plate, if lipid separation was to be achieved.



Figure 8-2: an illustration of a TLC chromatograms of lipid extract and standards eluted using hexane/diethyl ether/glacial acetic acid (70/30/3, v/v/v) (lane 1 = cholesterol standard, lane 2 = triacylglycerol standard and lane 3 = lipid extract) (A = non activated and B = activated TLC plates). The lipid extract was obtained from the activated sludge (Finham STW, batch 2).

(iii) Determining a suitable detecting method for lipid

It was necessary to develop a suitable method for visualising the lipids at the end of separation, for easy identification. This was done by firstly determining the optimum conditions: temperature, time and derivatizing reagent which could give black or brown spots of the lipid chromatogram in a white background using a destructive method, as described in Section 8.2.1.1.1 (3a). Secondly, the lipid chromatogram was visualised under a UV lamp using a non-destructive method, as shown in Section 8.2.1.1.1 (3b). The results obtained from both methods were compared at the optimum conditions. Thus, Figure 8-3 shows the chromatogram obtained by destructive method from four different TLC plates charred in the oven at 140, 160 and 180°C between 5 and 10 min, using 5% concentrated sulphuric acid in ethanol, as derivatizing reagent. The results show that palmitic acid was not visible at these conditions and that all other lipids

(cholesterol, vegetable oil, palmityl palmitate, mono-, di- and triacylglycerol) were clearly identified at 180°C between 5 and 10 min.



Figure 8-3: plates 1, 2, 3, 4 were charred at 140°C for 10 min, 160°C for 10 min, 180°C for 10 min and 180°C for 5 min, respectively (lane 1 = palmitic acid, lane 2 = cholesterol, lane 3 = glyceryl trioleate, lane 4 = lipid extract, lane 5 = mono-, di- and triacylglycerol and lane 6 = palmityl palmitate). The lipid extract was obtained from the activated sludge (Finham STW, batch 2)

Since palmitic acid was not visible at the conditions considered above (Figure 8-3), the charring time was increased (120, 110, 70 min) while using the same temperatures 140, 160 and 180°C, see Figure 8-4. The results show that a faint appearance of palmitic acid was observed at 140°C and 160 °C and more obvious spot was obtained at 180°C under the times considered (Figure 8-4).

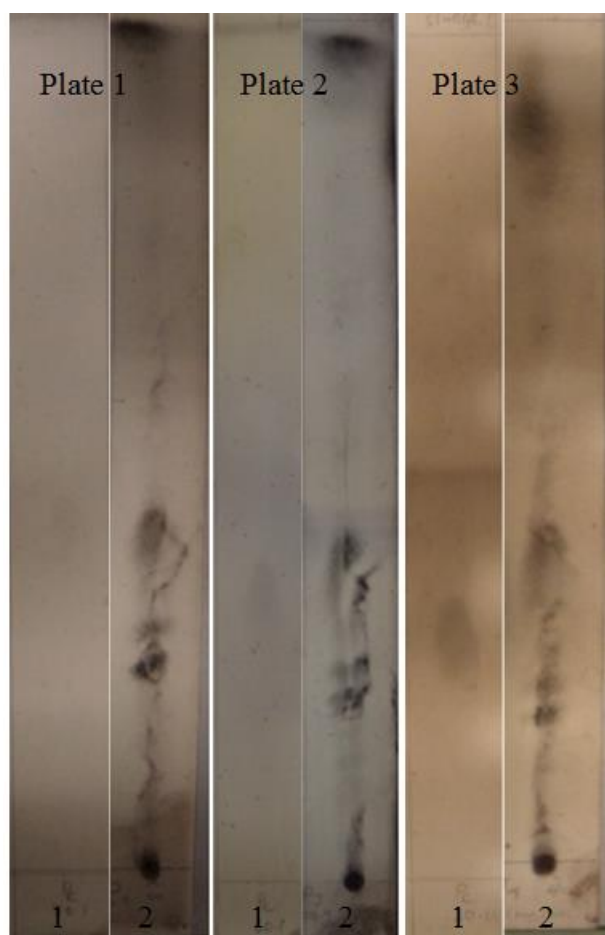


Figure 8-4: plates 1, 2, 3 were charred at 140°C for 120 min, 160°C for 110 min and 180°C for 70 min, respectively (lane 1 = palmitic acid and lane 2 = lipid extract). The lipid extract was obtained from the activated sludge Finham STW (batch 2).

The results obtained when the derivatizing reagents: 5% sulphuric acid (98%) in ethanol and 5% sulphuric acid (98%) in methanol were used to visualise phospholipids at charring condition of 180°C for 10 min are presented in Figure 8-5. This shows that brown spots of the phospholipids as expected were visible at this condition, when 5% sulphuric acid in ethanol was used (Figure 8-5, plate A) and were faint when the derivatizing reagent was prepared in methanol (Figure 8-5, plate B).

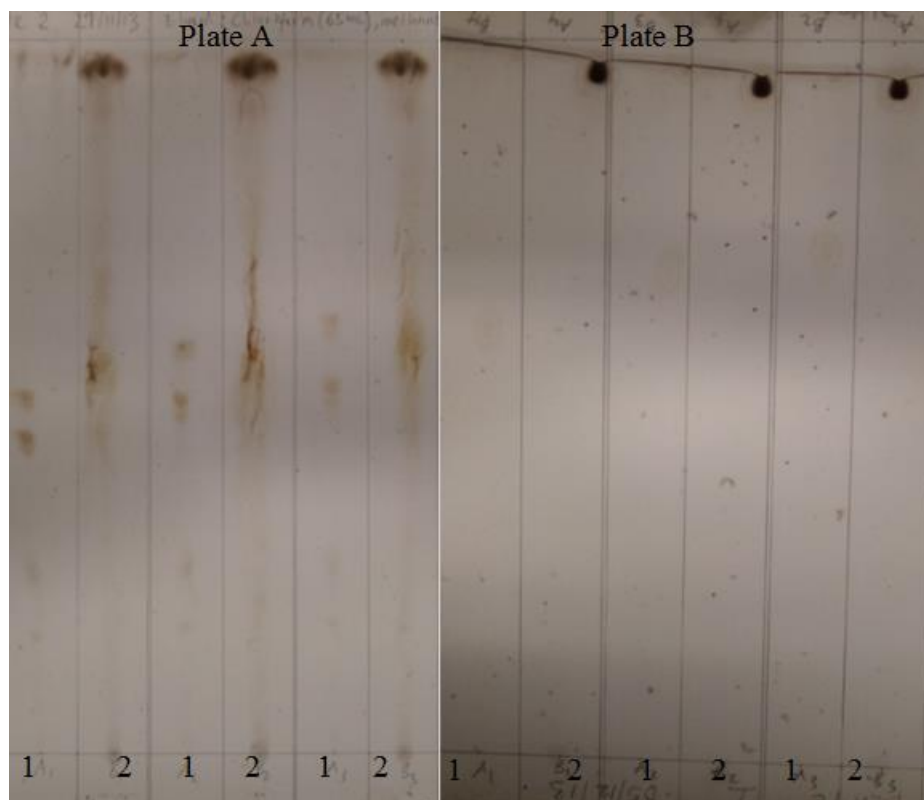


Figure 8-5: triplicate TLC runs per plate of phospholipid analysis for evaluating detection methods: Plate A - charred with 5% sulphuric acid (98%) in ethanol and plate B - charred with 5% sulphuric acid (98%) in methanol at 180°C for 10 min, respectively, (lane 1 = phospholipid standard mix and lane 2 = lipid extract). The lipid extract was obtained from the activated sludge Finham (batch 2).

Hence, lipids can be best visualised by TLC under the conditions considered using the destructive method with 5% sulphuric acid in ethanol as the derivatizing reagent at 180°C for 10 min. The non-destructive method using a UV lamp with a wavelength of 254 nm did not show any visible spots of lipids.

(iv). Choice of eluent or solvent Systems

It was necessary to determine a suitable solvent system which has the capacity to resolve most neutral and polar (phospholipids) at a single run, as the efficacy of TLC depends on the polarity of the solvents and lipid. The different solvents were mixed together using specific ratios in

order to either increase or reduce the polarity of the solvents, depending on the lipid class of interest, see Section (8.2.1.1.1 (4)). The choice of solvent system in separating all naturally occurring complex lipids depends on the lipids class of interest (Henderson and Tocher, 1992). In this study, neutral and polar (phospholipids) lipids were our interest. The results of the experiments show that neutral lipids were best resolved using petroleum ether/diethyl ether/acetic acid (80/20/1, v/v/v) and phospholipid was well separated with chloroform/methanol/28% ammonia (65/25/5, v/v/v) as the eluent solvents, see Sections 8.3.2 and 8.3.3. These solvent systems were used as the choice solvent for the neutral and polar lipid quantitative analysis. Although, according to Henderson and Tocher (1992), no single solvent system can completely resolve all the lipids classes in a complex lipids using a single elution.

8.3.2 *Identifying the neutral lipid in the activated sludge*

The lipid extract from activated sludge (Finhan STW, batch 2) was analysed using TLC, according to the method described in Section 8.2.1.1.2, this was done in order to determine its neutral lipid contents which might be available for bioenergy (biodiesel and renewable diesel) and oleochemicals production. The solvent systems and amounts of lipid loaded on the plate are presented, below. The neutral lipid classes were identified as sterol (cholesterol), wax ester (palmityl palmitate), acylglycerols (mono-and di-acylglycerol) and free fatty acid (palmitic acid), as shown below and in Figure 8-6. The lipids identified are designated with the first numbers while the second numbers denoted lipids standards, both separated by comma (Figure 8-6). For example, 5, 9 represented monoacylglycerol identified in the lipid extract using monoacylglycerol standards.

1. *Sterol (cholesterol), wax ester (palmityl palmitate) and monoacylglycerol*

The sterol, wax ester and monoacylglycerol were analysed using petroleum ether/diethyl ether/acetic acid (80/20/1, v/v/v). The amount of lipid extract applied on the plate was 400 µg and

that of the lipid standards were 152 µg, 238 µg and 200 µg for cholesterol, palmityl palmitate and acylglycerol (mono-, di- and triacylglycerol mix), respectively. The results are presented in Figure 8-6. Sterol (cholesterol) was identified at lipid spots 7, 10, wax ester (palmityl palmitate) at 1, 11 and monoacylglycerol at 5, 9. The acylglycerols (di- and triacylglycerols) were not identified using the solvent system. Hence, there was need to explore the efficacy of other solvent systems.

2. Diacylglycerol (1, 2-diacylglycerols and 1, 3-diacylglycerols)

The lipid extract was analysed for di- and triacylglycerol using hexane/diethyl ether/acetic acid (70/30/1, v/v/v), according to the method described in Section 8.2.1.1.2. The amount of lipid extract loaded on the TLC plate was 400 µg while that of mono-, di- and triacylglycerol standard mix was 200 µg. The result obtained indicated the presence of 1, 2-diacylglycerols and 1, 3-diacylglycerol (Figure 8-6). From Figure 8-6, numbers 14, 18 and 13, 17 denoted 1, 2- diacylglycerols and 1, 3-diacylglycerol, respectively. It was observed that there was no triacylglycerol in the activated sludge.

3. Free fatty acid (palmitic acid)

The free fatty acid in the lipid extract was identified as palmitic acid using hexane/diethyl ether/acetic acid (70/30/3, v/v/v). The amount of the lipid extract and palmitic acid standard applied on the TLC plate were 400 µg and 200 µg, respectively. The palmitic acid was identified at the spot 21 on the TLC plate corresponding to the lipid standard 23, as shown in Figure 8-6.

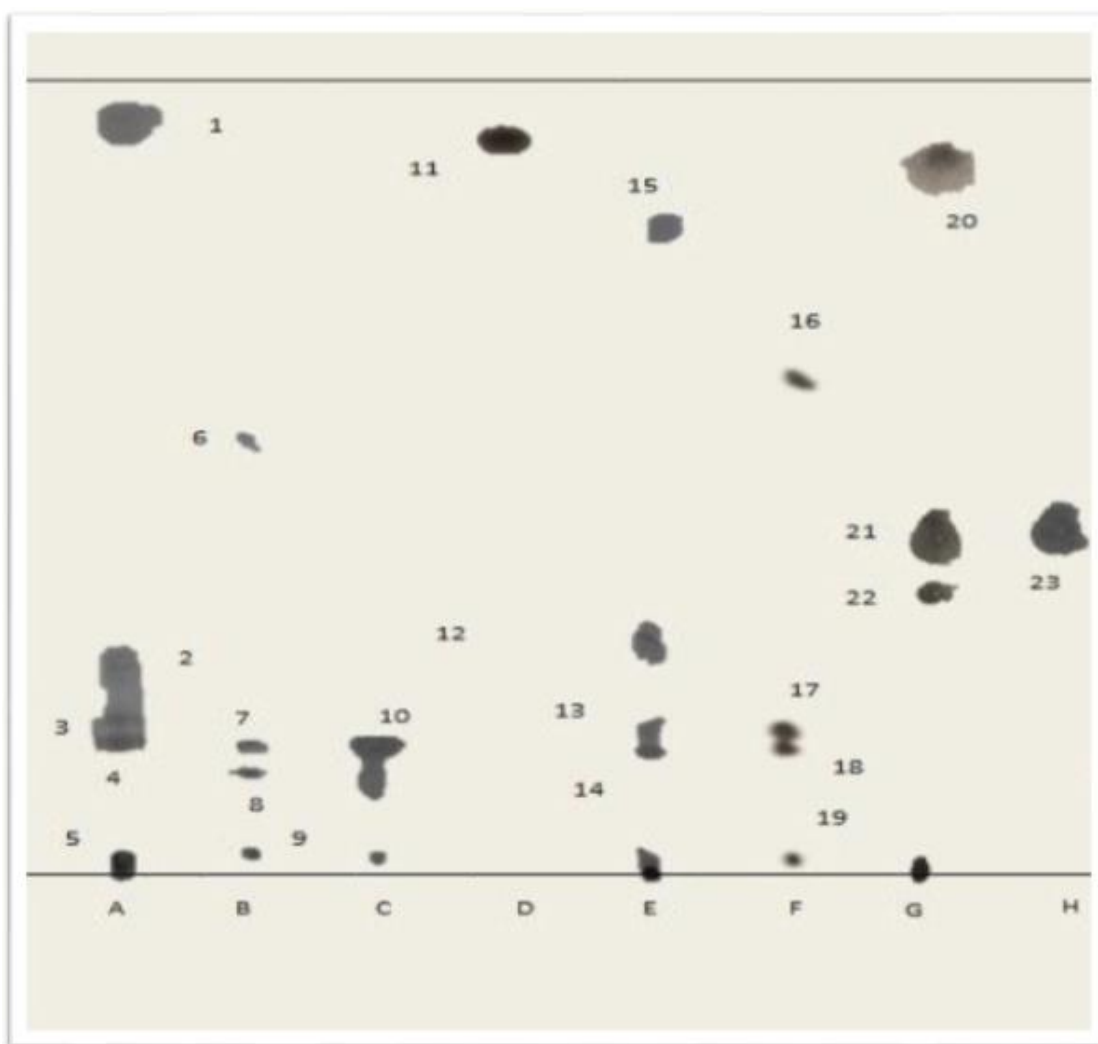


Figure 8-6: determination of neutral lipid composition in activated sludge (Finham, STW, batch 2) by thin layer chromatography (lanes A, E and G = lipid extract sample, lanes for standards B = mono-, di- and triacylglycerol mix, C = cholesterol, D = palmityl palmitate, F = mono-, di- and triacylglycerol mix and H = palmitic acid). Cholesterol, palmityl palmitate and monoacyl glycerol were analysed using petroleum ether/diethyl ether/acetic acid (80/20/1, v/v/v, see lanes A-E), 1, 2-diacyl glycerol and 1, 3-diacyl glycerol were developed using hexane/diethyl ether/acetic acid (70/30/1, v/v/v, see lane F) and palmitic acid was eluted using hexane/diethyl ether/acetic acid (70/30/3, v/v/v, see lanes G-H). (7, 10 = cholesterol, 1, 11 = palmityl palmitate, 5, 9 = monoacylglycerol, 14, 18 = 1, 2-diacylglycerol, 13, 17 = 1, 3-diacylglycerol, 21, 23 = palmitic acid). Spots 2, 3, 4, 12, 15, 20 and 22 are suspected lipids while 6 and 16 are triacylglycerol standards

8.3.3 Identifying the phospholipids in activated sludge

The presence of phospholipids in activated sludge was assessed, for possible bioenergy (biodiesel and renewable diesel) and oleochemical production, by TLC, according to the

method described in Section 8.2.1.1.3. The results are expressed in terms of the retention factor (Rf) (Table 8-2). They show that phospholipid standard mix were not eluted using chloroform/methanol/acetic acid/water (25/15/4/2, v/v/v/v) and chloroform/methanol/acetic acid/water (50/25/7/3, v/v/v/v), although, these solvents system resolved the lipid extract into three and one spots of unknown lipid, respectively, see Table 8-2. While chloroform/acetone/methanol/acetic acid/water (6/8/2/2/1, v/v/v/v/v) resolved the phospholipid standard mix into two spots of lipids with Rf values 0.73 ± 0.04 and 0.978 ± 0.004 , respectively. Chloroform/methanol/28% ammonia (65/25/5, v/v/v) (Rouser et al., 1970) was found to resolve the phospholipid efficiently and was the preferred eluent system (Table 8-2). The phospholipid found in the activated sludge were PE, PC, PI and LPC. These lipids can be used as a feedstock in bioenergy (biodiesel and renewable diesel) and oleochemical production.

Table 8-2: identifying the phospholipids in activated sludge (Finham STW, batch 2) using retention factor (Rf) obtained from TLC

Solvent system	Retention factors (Rf) of lipids								
	Lipid standard (phospholipids mix)				Lipids extract				
	PE	PC	PI	LPC	PE	PC	PI	LPC	Unknown
Chloroform/methanol/ acetic acid/water (25/15/4/2, v/v/v/v)	-	-	-	-	-	-	-	-	0.88 ± 0.01 , 0.91 ± 0.01 , 0.99 ± 0.00
Chloroform/methanol/ 28% ammonia (65/25/5, v/v/v)	0.54 ± 0.02	0.45 ± 0.02	0.24 ± 0.01	0.16 ± 0.01	0.55 ± 0.02	0.44 ± 0.01	0.20 ± 0.00	0.15 ± 0.04	0.975 ± 0.01
Chloroform/methanol/ /acetic acid/water (50/25/7/3, v/v/v/v)	-	-	-	-	-	-	-	-	0.98 ± 0.00

(No known phospholipid was identified using chloroform/methanol/acetic acid/water (25/15/4/2, v/v/v/v) and chloroform/methanol/acetic acid/water (50/25/7/3, v/v/v/v))